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Howland Memorial Number

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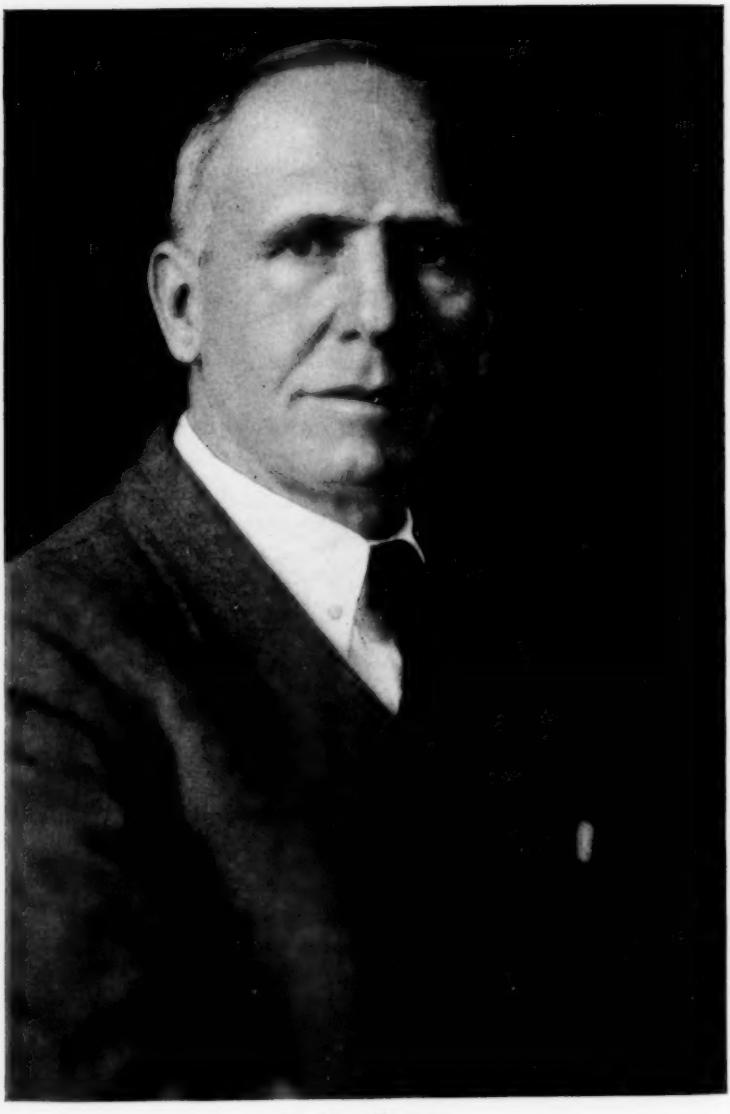

DR. JOHN HOWLAND

MEMORIAL MEETING FOR DR. JOHN HOWLAND

On January 30, 1927, at the Engineering Building at Homewood, many friends and associates attended a memorial meeting for Dr. John Howland, late Professor of Pediatrics in the Johns Hopkins University School of Medicine, who died in London, England, June 20, 1926.

Dr. Frank J. Goodnow, President of the Johns Hopkins University, presided, and Dr. William H. Welch, Dr. Simon Flexner, Dr. William G. MacCallum and Dr. Graham Lusk spoke of Dr. Howland's life and his contributions to medical science. These addresses follow:

President Goodnow:

We have met this afternoon as you know to pay tribute to one of our colleagues, a man who endeared himself to all of us as a most loyal friend, a most genial companion, and a most successful teacher and investigator. His loss leaves a gap in the ranks of the teaching force of the University which will be exceedingly difficult to fill. Others can speak of him much more effectively than I can and they are here to speak briefly on the various phases of his life and his work.

Dr. Welch will speak in the first place in regard to the work of Dr. Howland.

Dr. William H. Welch:

It was suggested that I should speak more particularly about Dr. Howland's work in creating the clinic for Children's Diseases at the Johns Hopkins Hospital. Other phases of his life and work will be touched upon briefly by other speakers. Extraordinarily vigorous qualities of mind and of body, coming from such an ancestral stock, assured a certain soundness of mind, robustness, and sanity of judgment. In his college days he fell in with the spirit of Yale of the time. He became one of the most popular and influential men in his class, interested in athletics, standing well in his studies, but not greatly stirred to scholarly interests and pursuits. As so often happens, these scholarly interests were stimulated when he entered upon his professional studies. At that time the best training that could be obtained was from the hospital service, and his two years as an interne at the Presbyterian Hospital, after receiving his medical degree from the New York University in 1897, probably formed the most important part of his professional training. Most significant of all was the year he spent as an interne

in the Foundling Hospital in New York where he first came in contact with and was associated with Dr. L. Emmett Holt, because at this time he was led into the field of children's diseases. The strongest professional influence during this mental period came from Dr. Holt, not only when he was associated with him as an interne, but when, on his return to New York, after a year's study in Berlin and Vienna, he became assistant to Dr. Holt, and to him I am sure Dr. Howland always felt the debt which a young man feels toward an older man in his career.

His ten years' work in New York City was a good introduction into research; he became an excellent clinician; he became interested in education; he became interested in the development of clinics; he made his mark particularly in children's diseases at the Bellevue Hospital. From the beginning he showed an investigative spirit. His early work on dysentery brought him into relations with the clinical, bacteriological and pathological aspects of a very important disease.

He was called to the Chair of Pediatrics at Washington University to organize a pediatric department, and entered upon a year's duties with probably the best pediatric clinic of that time. He was there only one year. Before that he had had the benefit of study in Czerny's clinic in Strasbourg, which represented probably the most important training from German sources which he received.

When we were ready to establish a clinic for children's diseases at the Harriet Lane Home after Dr. von Pirquet had returned to Vienna, we were naturally eager to find one who was best qualified for this position. Dr. Holt advised us that we could not possibly find a better man for the position than Dr. Howland, and he was induced to come here. I should like to emphasize particularly that he created here in the Johns Hopkins University the first University Pediatric Clinic worthy of the name in this country. He brought to this position ideal qualifications. He was an excellent clinician; a good teacher, greatly interested in the advancement of knowledge in this subject. He realized that the time had come for a great improvement and reform in the organization, conduct and teaching of clinical medicine in this country. He was prepared to devote his whole time, energy, and thought, the best that was in him, to this end. That constitutes a contribution to medical education of the very first order—a contribution with which his name will always be associated as the pioneer. He realized the opportunity which was presented to him. He was thoroughly in sympathy with the University idea of the clinic. He had some advantages in that he practically had the opportunity to organize the clinic from the beginning and to carry out his own ideas. I say he was an excellent clinician. His first thought was always the care of sick infants and children. Those who had the opportunity of hearing Dr. Howland at his last public appearance at the meeting of the Half Century Fund at the Belvedere Hotel will recall his remarks upon the importance of the best possible care of children based on the best knowledge. He was an excellent teacher. He had that great quality of attracting to him not only students, but especially his staff, and those who desired to enter this very interesting field. Those who hold today the most important positions as the heads of Pediatric Departments of

Harvard, St. Louis, Yale, and other important medical centers in this country, with clinics modeled after the one he established here, were nearly all trained under Dr. Howland. No one could have been more alive to the importance of increasing knowledge in the field in which he was interested. He was of an investigative type of mind and the outlook in the way of scientific investigations from his clinic was a wide one.

I would like to dwell upon the great contribution which he made as the first to establish a full time University clinic in this country. He was highly sympathetic with the ideas of a full time service in the University and the foundation of this great clinic exemplifies perhaps as no other did the advantages of that system.

We are all interested in the advancement of medical education and we all feel a great debt of gratitude to Dr. Howland who rendered an unforgettable service. His loss is an irreparable one. Others will dwell upon his qualities as a man. He was a most delightful companion; a man of extraordinary sanity. He had very definite aims and principles to which he adhered strongly and also a certain New England firmness, I think, in his adherence to what he believed to be a sound principle. He was one who rendered a very great service to this University and one whose memory will always be cherished in this University.

Dr. Simon Flexner:

I shall speak briefly of my connection with Doctor Howland, which began in 1905, or at the time when the Rockefeller Institute was opened in New York. Doctor Howland was at that time associated with Doctor L. Emmett Holt and was pathologist to the New York Foundling Hospital and an attendant at the dispensary of Bellevue Hospital.

I was first brought into contact with Doctor Howland in a strictly personal way. Doctor Holt assigned Doctor Howland to care for my own baby, a connection which proved sympathetic and professionally fruitful.

Doctor Howland was an excellent clinician, but that is something you have already been told. We were fortunate in quickly forming a real friendship, through which I learned of his unusual attitude toward the practice of pediatrics. He was convinced that the career of a practising clinician as then pursued in New York would not content him. While the mere practice of medicine made him restless, he had found satisfaction in following the clinical cases into the pathological laboratory where the effects of disease could be studied objectively. It was the pathology of disease, using that term in a broad sense, which chiefly occupied his mind.

It was obvious to everyone that Doctor Holt entertained a high opinion of Doctor Howland's ability, and I think it is safe to conjecture that, had Doctor Howland remained in practice in New York, he would have been an outstanding clinician ranking alongside his master, Doctor Holt.

But as often happens with men of his temper, he was possessed by a demon which kept him restless and made him discontented with the practice of medicine

as usually pursued. It was in his nature to reach out for and grasp opportunities. The time happened to be just ripe for the study by bacteriological methods of the gastro-intestinal diseases of infants. In this way, he was led into the study of dysentery in children. He was fairly equipped to deal with the intricate subject of intestinal diseases, and where he lacked training, his ingenuity and industry soon provided the needed technique. He was one of those who brought order into that almost chaotic group of diseases of children.

The personal relationship which I have mentioned continued, and as a result, Doctor Howland who had become impressed with the cases of sudden death in children, associated with the condition called "status lymphaticus," wished to make studies of the pathological state found at autopsy, namely, enlarged lymphatic glands and the persistent thymus.

It was decided that he should carry out experiments at the Rockefeller Institute. This was in the early days of investigation of the so-called cytotoxins, or specific cell poisons. The plan was to prepare specific cytotoxins for say the thymus gland, and with them attempt the reproduction of "status lymphaticus" in animals.

The research never really got anywhere. It was impossible to approach the solution of the problem from that gateway. Nevertheless, the undertaking taught certain things and, most of all, it taught Doctor Howland a technique which in one or another form proved to be very useful in the succeeding years in the study of the immunity phenomena.

It was characteristic of Doctor Howland to seek to extend his knowledge and technical equipment. In this way, one and then another field would be entered, the new technique acquired and a new subject studied.

There is a logic in the way openings come in science—openings by means of which new knowledge is gained. Those who take advantage of these openings often have a way of seeing and seizing opportunities, often called flair, which to the uninitiated seems almost uncanny. Doctor Howland proved to possess this insight and because of it and his technical competence, his clear mind, his clinical ability and his good sense, he was enabled to advance pediatrics in the United States, and in a comparatively few years, in an altogether remarkable manner.

The acquaintance begun so auspiciously more than twenty years ago in New York, ripened and deepened with the passing years into a solid structure of friendship, and on my part of admiration for the man who so well filled the several offices of clinician, teacher, and investigator. Doctor Howland, whose sterling worth and winning personality we all felt, came to occupy an outstanding position in pediatrics, not only in the United States, but in the world at large. Through his contributions to knowledge, the students he trained and fired with high ideals, and his own splendid and endearing personal qualities, Doctor Howland will continue to live in the history of the Johns Hopkins Medical School and the annals of medicine in this country.

Dr. William G. MacCallum:

It is my part to speak of John Howland's work and of his place in this community and this University.

In our grief over the loss of one whom we miss so much it is still a pleasure to speak of his really good work and to think of its effect on future thought along those lines that he followed. And it is a pleasure, too, to watch, in the careers of those men whom he picked out to be his assistants, the lasting effect of his influence and his example.

The details of his own story have been told many times. He served as interne in the Presbyterian Hospital and in the Foundling Hospital and afterward went to Europe for a year's study. When he returned he became associated with Dr. Holt as instructor and associate in Pediatrics in the College of Physicians and Surgeons of Columbia, and was connected as one of the assisting attending physicians with several hospitals, notably the New York Foundling Hospital, where for nine years he was pathologist as well. That remarkable place must have afforded him the material for much of his clinical and pathological experience. Then he became head of the children's clinic at Bellevue but two years later he accepted the professorship of Pediatrics at the Washington University in St. Louis. Before going there he spent a year in study with Czerny in Strasbourg. He remained only a year in St. Louis and then came to Baltimore in 1912 as the successor of von Pirquet.

At first, when he was working at the Foundling Hospital after his student year in Vienna, he was interested in the pathological anatomy of the diseases he encountered in the infants. The bacterial causes of dysentery in their several types had recently been discovered and he made a study of the intestinal changes produced by one of these. There were other clinical and pathological subjects at that time too that stirred his interest enough to have made him publish several papers—one on appendicitis in infants, another on chills in typhoid fever and somewhat later two on the symptoms and pathological anatomy of the so-called status lymphaticus.

But then he began to feel the importance of chemical studies if there were to be any prospect of a complete understanding of the nature of a disease. He must know what chemical disturbances in the body caused the symptoms and were related to the structural changes, for he felt that no amount of watching of symptoms or contemplation of post-mortem alterations could afford the ultimate explanation of what was happening. Whether he deliberately arrived at that conclusion then, it is impossible to say, but at least it was the beginning of the habit of the rest of his life to approach his problems from the chemical point of view. But then it is also true that his flair for chemistry was doubtless the reason for his preferring problems which could be solved by chemical studies to those in which mechanical and functional disturbances were alone to be found, or those in which the chemical solution is still far beyond human knowledge.

At this time, then, he became interested in the rather violent symptoms of

poisoning that appeared in the recurrent vomiting of children and with Dr. A. N. Richards he searched for some clue to its nature by studying various poisons such as indol and chloroform. These studies at least resulted in the first clear recognition of the profound destruction of the liver that follows poisoning by chloroform, even when it is inhaled.

It is perhaps an example of his attitude at this time, when his enthusiasm for the chemical approach to difficult problems was rising, that he studied those fatal cases of diphtheria in which the heart was supposed to be paralysed, but finally agreed with other workers that it was really an effect upon whatever part of the brain maintains the tone of the blood vessels. He planned chemical studies and indeed did publish a preliminary report, but it was not very satisfactory, for that seems to be one of the cases which our chemistry is as yet hardly adequate to solve.

Howland was not by early training a chemist and he realized the enormous difficulties in the way of acquiring sufficient mastery of that science to use it with confidence and precision. It is something like an attempt to master the violin in adult life. But he set himself doggedly at it and in the end I think he succeeded, although he never really trusted himself alone but engaged the interest and assistance of the best chemists he could find.

It must have been just after this that he began to work with the calorimeter upon the chemistry and energy production of the body of the normal child. This was a development and rounding out of his chemical attainments and his papers in this field show a new familiarity with exact chemistry and a new inventiveness which expressed itself in apparatus and methods.

Such studies led him at once to a more critical consideration of what is necessary for the proper nutrition of infants and all the need for quantitative accuracy as well as the need for correct interpretation of what was wrong when the babies did not thrive. When he came to lecture before the Harvey Society in 1913 on the artificial feeding of infants we find that uninviting subject turned into a fascinating history of the trials to which babies must have been subjected in olden times, their phenomenal powers of survival, the basis for a proper, clean, and correctly adapted diet and above all an honest recognition of the problems and difficulties that remain.

He was still interested in the fatal poisoning of those babies with recurrent vomiting and diarrhoea and perhaps was stirred by Czerny to the idea that this was a form of acidosis. Accurate work on the acid-base equilibrium in the body was new at that time and the really effective methods of study were yet to be devised, but Howland plunged into it with Marriott and together they invented new methods and quickly adopted those of others. The extraordinary opening out of a field of study that comes of itself with the invention of new methods must have delighted him, for he worked for several years in this field and discovered many things of which perhaps the most important were that the intoxication in children does not depend especially upon the formation of acetone bodies, and

that the inability of the kidney to excrete acid phosphates might upset the acid-base equilibrium. But these studies gave him an understanding of this complex subject of the acid-base equilibrium which allowed him later to write on it with the most satisfying clarity.

Then he began to interest himself in rickets of which he saw so much among the negro children of this town and once more he felt that no amount of contemplation of the structural changes in the bones, nor the deformities, nor the symptoms, could explain anything and that one must know what chemical constituents of the food or the blood or the bones were deficient or unbalanced—and why these factors should cause the softness of the bones and their bending and bulging in all directions. For the rest of his life he labored over this—and all his men with him and others too who lent their special skill. He and Marriott and Kramer and Tisdall devised ever new methods for measuring calcium and phosphates and other constituents of the blood in such minute quantities as could safely be taken from these babies for study. That as usual was a key for the opening of this locked problem and they found that it was not merely the diminution of lime salts but particularly the lowering of the phosphorus in the blood that made the deposit of hard calcium phosphate in the bones impossible. This was a signal discovery and it has been confirmed again and again. They found that the tetany or spasms that often accompany rickets appeared when the lime salts were greatly decreased and brought into common use the giving of calcium to these children which will stave off the spasms as long as it is given.

McCollum and his assistants had devised a diet deficient in a particular vitamine which they discovered, upon which rats soon developed rickets, and Howland, and especially his assistants Park and Shipley, took part in this work and made most interesting studies of the progress of the chemical changes in the blood and tissues of these rats and the structural changes in their bones and further in the wonderful cures that can be brought about with cod-liver oil and ultra-violet light.

It seems that this tenacious study of rickets with all the forces at his command is Howland's best work and in his second Harvey Lecture he has presented it in another fascinating historical narrative which runs on into an account of all his chemical and experimental studies which have so wonderfully contributed to a clear understanding of this complicated disease. Once more with characteristic honesty he presents the difficulties and doubts that remain.

Such was the work that he had time to do. It is a contribution to the sum of our knowledge greater than most men can make and it was made not through mere flashes of genius but through sheer determination to learn the truth about those things which filled his mind.

Except for his lectures and addresses Howland seldom published any paper alone. In all his work he associated with himself younger men, chiefly those who could contribute some special knowledge. And he had special ability to choose his assistants and to inspire them with eagerness to work. That his was the

inspiring intellect is very clear from the character of the subjects and of the work itself, for almost all of the numerous papers that came from his clinic are concerned with problems that interested him and most of them were carried out in direct connection with his own work.

It was a very productive time as is shown by the four thick volumes of papers since collected and bound together for the library. It would take too long to discuss them all, but I may mention the work of Park on the thymus and many clinical topics, of Park and Shipley with others on the experimental study of rickets, of Marriott, Kramer, Tisdall and others on various chemical aspects of acidosis, rickets and tetany and of Gamble, Ross and Holt on similar subjects. Rather apart from the general trend of the work was that of Blackfan with Dandy on hydrocephalus and that of Rivers, Davison and others on various bacterial infections.

Such activity, of which only a part has been suggested, could have come only from an enthusiastic, interested group with a recognized leader.

That he was either a capable judge of men so that he chose the most able, or else one who could reconstruct and train less independent spirits, is evident from the fact that so many important university posts in Pediatrics are occupied by his former assistants who, one may be sure, are carrying on in their new places the traditions that he established, and are working and teaching with much of the attitude toward their clinical and laboratory problems that he imparted.

Although Howland was by no means eager about details of administration of the hospital, he did succeed in attaining an admirable organization with an effective staff devoted to the study and care of the children in the wards and to the proper conduct of the large dispensary which is part of that department and not of the general dispensary. He himself felt, I know, that the care of the sick children stood first in the programme of his responsibilities and he was unremitting in his attention to the wards and in assuring himself of the competence of all his staff to whom a part of this responsibility had to be delegated. It was a source of regret to him that for so much of his time they had not the funds to open the hospital to as many of the sick children as they might have cared for.

Howland was a gifted clinician, gifted especially with sane judgment of what was probable, together with an extraordinary analytical memory of his long experience. The diseases that occur are not endless in their variety and repeat themselves with changes only in detail, but if experience is to be useful, it must be the memory of cases that have been really studied and the conclusions verified by the aid of pathology, and not, as is so often true, merely a long series of surmises which are never corrected. He could never have been content with anything so superficial and his knowledge of disease was real and completely at his command. His staff learned from him something new every day from this store but more valuable than that was what they learned of his attitude toward the daily problems that they met together. He gave much of himself and stirred not only their

loyalty and admiration but their enthusiasm and their desire to put their whole energies into their work.

He loved to teach and the students went eagerly to his clinics which were generally devoted to the demonstration of the outstanding features of several cases rather than to a minute study of one. He presented a subject with the utmost clearness and with an enjoyment that was enhanced by his way of bringing a student into the discussion with questions that were obviously intended to show what humiliating mistakes might be made. But it was a very friendly sense of humor and they enjoyed it as much as he did. They admired his honesty in admitting at once that he did not know, when some difficult question arose, for they are quick to draw a sharp line between enquiring minds and those that are dogmatic.

One felt at once with Howland that his was a most forceful personality and that perhaps the strongest elements of this were his courage and his indomitable will to succeed in whatever he set his hand to do. In his earlier days, when he was a distinguished tennis champion, it was not so much sheer brilliancy as this iron determination that nothing could weary or dismay that brought him victory against great odds. And later in his medical work it was the same. He was determined to triumph over the difficulties that he met in the laboratory and by persistence and untiring work he succeeded and succeeded brilliantly. This too came through a mastery of technique, sustained and intense interest and great tenacity of purpose.

It was not at all inconsistent with this great strength that he was very tender with little children—they seemed to know the way to his heart and one is sure that he felt suddenly softened by their little appeal. I have known the mothers of some of them and they tell me that, when he came, at once the children knew him and reached out to him and they themselves were perfectly at rest and confident that now they need no longer be anxious.

It was not that he had artifices to amuse them but just his human sympathy, and one feels that this was there before he chose his career and perhaps without his knowledge really decided it for him.

He was a most wonderful companion and I remember with especial pleasure some days that I spent with him and Gamble driving down through the western shore of Maryland. His interest in everything—the gardens, the quaint old houses, the relics that told the history of a century ago and especially the entertaining and talkative characters that we met—remains in my memory more vividly than the places themselves. We always intended to take other trips like that but there never was time.

If we try to sum up his service to this University it will doubtless seem that the most prominent thing is his organization of the Harriet Lane Home for Sick Children, and, indeed, although it was opened before he came, it was he who made it what it is. But it seems that greater than this was his personal influence in making this clinic the place to which men wished to come from all countries for

instruction and inspiration and above all to be with him. He was the commanding figure in Pediatrics in this country and set a standard toward which the others strove without envy. And in his brief time he trained a number of men who will carry on for him. Howland had spent all his life in touch with men of intellectual prominence and his own logical mind had developed with this background. It had a judicial cast and he arrived at definite convictions which were singularly fair and most palpably honest. He had a penetrating understanding of human motives and a keen sense of humor, which are two elements that contribute to wisdom in the ordering of the affairs of a medical school, and his counsel, and especially his estimate of men, was always sought and most highly valued.

He was a distinguished member of this University, a productive investigator, an experienced and able clinician, an inspiring teacher and leader—a wise and honest colleague.

Dr. Graham Lusk:

John Howland was the direct descendant of another John Howland who, on the 21st of December, 1620, disembarked from the *Mayflower* at Plymouth, thereby becoming one of the original settlers of New England. The John Howland of our day, who was our friend, represented a type of individual born with a heritage of struggle and labor, of conscientious devotion to righteous causes, of scrupulous regard for the rights of others and of the desire for worthy accomplishment. Together with this common New England background he had a fine mind, more than usually endowed and incessantly active, a mind full of ideas, driven by the inherited spirit of the pioneer and driven especially in the intellectual field. His nature was the product of New England maturity. The struggle against adverse forces was there, but it was of an intellectual, no longer of a physical character.

I met him first when he was a college student at the home of Dr. Sanford, of New Haven, who afterward became his father-in-law. As a sophomore at Yale he made up his mind to study medicine and arranged his life to that end with a singleness of purpose characteristic of him.

Whatever he did, he did well. He concentrated upon certain activities, and this very singleness of purpose made him a formidable adversary. In sports he was what professionals call "a good performer;" he produced his best when he was put to the test. In a great tennis match at Narragansett with the English player, Goodbody, who was his equal in skill if not his superior, he wore down his opponent in an exhausting five-set struggle. And again, when he was working in pediatrics in Vienna with von Pirquet, he entered the lists against another British player, Ritchie, then champion of Austria. The Englishman won the first two sets and then went down in defeat before the aroused force of John Howland's power, a power which always grew stronger in times of adversity. Pertinacity and determination are terrible in an adversary, not only in the field of mimic conflict, but in life as well.

But there was nothing terrible or terrifying about his nature. He wished to like people and therefore people liked him. He was completely free from pretense and was direct and simple in his human contacts. Among his intimates he was boyish, unrestrained, with an infectious capacity for enjoyment. Though he was an intellectual, in some ways exactingly so, he was democratic in his human approach to the point of simplicity. He was cordial without being effusive; open without cheapening himself.

He fully realized that no one is or ever can be universal in approach, contact and understanding. Without austerely secluding himself he associated his energy and his personality with those situations and with those types of persons with which he felt himself to be most at home. Without detaching himself from the common life, he had and he knew he had the integrity of his own type—a priceless element in character and the creator of power.

As a medical student in New York he had been trained in a medical atmosphere in which no note had been struck except that of commercial gain. His nature was too great to accept the latter as more than an incident, not the main object of life.

His difficulties at St. Louis were due to his New England conscience. His soul rebelled when he was reproved for admitting two colored children into the wards of his hospital. It could not have been otherwise with him. This was only one of his troubles. When he later resigned his position he insisted upon returning to the University the money given to him for his trip abroad.

An old time physician has written, "There is nothing greater or more beautiful in the world than man. He is the mightiest and most elevated example of thought and education. His existence, his struggle, his suffering are all in the highest degree wonderful and stimulating."

This is the picture one may draw of Howland at that time.

The spirit of the frontiersman of the Seventeenth Century which did battle with the forces of nature was transmuted in him into a spirit of the intellect capable of leading others through the sometimes turbulent but often tranquil waters of scientific thought and action. The mental process was direct, precise, independent. And this was the reason why he found peace and rest for his soul at the Harriet Lane Hospital. He had one great temptation, the offer of the chair of pediatrics at Harvard. The appeal to his New England character was strong. He told me he felt he could speak to everyone standing on a street corner in Boston and be understood by them. But he felt that acceptance would have meant the loss of three precious laboratory years from his life, and so he resolved to remain in Baltimore. The last time I saw him, about a year ago, he told me that the arrangement of his life was the only one suitable for his tastes and that he was perfectly happy in it.

At the last meeting of the Board of Directors of the Russell Sage Institute of Pathology a minute was adopted setting forth that he had been a "faithful fellow-member of the Board." His letters were often signed "Faithfully yours." The outstanding beauty of his life was comprehended in the word *faithfulness*—faithfulness to every duty in life and to every friend he had.

A STUDY OF THE INORGANIC CONSTITUENTS OF THE SERUM IN CHILDREN WITH ACUTE NEPHRITIS[1]

KENNETH D. BLACKFAN AND BENGT HAMILTON

From the Medical Clinic of the Infants' and Children's Hospital and the Department of Pediatrics, Harvard Medical School

The forms of nephritis most commonly seen in children are the acute glomerular and the acute tubular nephritis. Chronic nephritis is relatively rare. In the majority of the acute types it is possible to postulate with a certain degree of accuracy the character of the process in the kidneys from the history of onset, the clinical symptoms and the laboratory findings. In acute glomerular nephritis there is a history of an acute infection, with haematuria and slight or moderate oedema following after several days. The blood pressure may be increased and the non-protein nitrogen in the blood may be higher than normal. Eclamptic uremia occasionally occurs in the more severe forms and may lead to the death of the patient. In acute tubular nephritis (nephrosis), the onset is insidious, oedema often being the first evidence of disease and usually developing to a marked degree. The urine contains a large amount of albumin, but no blood. The blood pressure is normal and the non-protein nitrogen in the blood is not increased. Recurrences are common and death, when it does occur, usually results from secondary infections. Uremia is rare.

During the past three years we have been making observations on children with acute glomerular and acute tubular nephritis. The purpose of this communication is to present data relating to the inorganic constituents in the serum in these two forms of nephritis. We have been especially interested in the amount of fixed base in the serum and we have made use of the Fiske method for the determination of this factor. By this method a value is obtained which gives the sum of the four bases: sodium, potassium, calcium and magnesium; expressed in cubic centimeters of 0.1 N base.

[1] Received for publication August 15, 1927.

METHODS

The determination has been made on serum; the blood was delivered through a small-bore glass tube under oil into a 15 cc. centrifuge tube, which was filled to the top and closed with a one-hole rubber stopper, permitting the expulsion of the superfluous oil; the hole was then closed with a glass plug. The chlorides were determined on 1 cc. of serum by the method of Fiske and Lin (1). The bicarbonate measurements were made according to Van Slyke (2) on 1 cc.; or, occasionally, 0.5 cc. of serum. The inorganic phosphates were determined by the method of Briggs (3). The total fixed base, as mentioned above, was determined by the Fiske method (4) on 1 cc. of serum. The calcium was precipitated as in the Kramer method, but always left standing over night; it was then centrifuged and

TABLE I

Children without nephritis

Values in cubic centimeters of 0.1 N in 100 cc. of serum*

CASE	DIAGNOSIS	Cl	HCO_3	PO_4	TOTAL ACID	TOTAL BASE	UNDETERMINED ACID	PROTEIN
H-n	Normal	103	27	1.6	132	156	24	
D-e	Normal	101	26	2.5	130	161	31	7.6
G-st	Normal	104	29	2.2	133	162	29	7.6
G-le	Normal					157		
G-nd	Normal	102	27	2.8	132	158	26	7.3
M-io	Enuresis	104	19			159		
B-ey	Asthma 3 weeks after attack	99	25			156		
C-11	Pharyngitis					158		
B-5	Postural albuminuria	105	24			159		7.8

* The valency of the phosphorus in the plasma has been taken as 1.8 (Henderson).

washed in the usual manner, but instead of the final determination by permanganate titration, the precipitate was transferred with four 2 cc. portions of water to a platinum crucible, the water evaporated on the steambath, the precipitate ignited, the CaO dissolved in 1 cc. of 0.015 N HCl and titrated back with 0.01 N NaOH (methyl red as an indicator). This method was suggested by Dr. Fiske. The protein was determined in the serum by micro Kjeldahl. No correction was made for non-protein nitrogen.

Table I gives data from nine normal, or practically normal, children. Of chief interest here are the values for total fixed base, which, as may be seen in the table, vary between 156 and 161 cc. 0.1 N base in 100 cc. of serum. The remarkable constancy of the total base in the serum

has previously been demonstrated in a study by one of us (5), where in 17 patients with epilepsy the total base in the serum was found to be between 156 and 161 cc. 0.1 N in 14; while in three, values of 153, 154 and 155 cc. were found. Bulger, Peters, Eiseman and Lee (6) place the normal limits of total fixed base in the serum as 150 to 165 cc. 0.1 N. We consider these limits somewhat too wide and in the

TABLE II

Tubular nephritis

Values in cubic centimeters of 0.1 N in 100 cc. of serum, except total electrolytes, which are expressed in millimols per kilogram of serum water

CASE	Cl	HCO_3	PO_4	TOTAL ACID	TOTAL BASE	UNDETERMINED ACID	Ca	PROTEIN	TOTAL ELECTROLYTES	NON-PROTEIN NITROGEN
Lever	104	19			143		4.0	3.9	278	
Greenburg	100	20	3.5	124	149	25		3.9	281	
Greenburg	108	18			143			4.1	281	
Crowell	105	19	2.4	126	153	27	4.1	4.7	290	
Petersen	102	21	3.8	127	143	16	3.8	3.8	278	
Petersen	107	17	3.2	127	144	17	3.8		280	
Nicholsen	105	9	3.6	118	138	20	4.0	4.3	253	
Pfeiffer*	99	20	4.2	123	143	20		4.2	274	
Wellducki*	104	25	2.6	132	152	20		5.2	295	
DeMattial	102	22			145		4.6	3.7	280	
Lightfoot	104	16	2.7	123	143	20		4.3	274	
Foley	104	23	3.9	131	161	30		4.3	301	
Trefrey	105	25	2.6	132	160	27		5.4	305	
Gardner	105	23	2.9	131	156	24		4.6	298	
Licharai	98				155		3.9	6.3		
Blanchard	97				151			3.3		
Perrie	106	20	2.6	129	139	10	3.3	2.8	275	30
Podget	99	25	3.0	127	145	18	3.4	2.6	284	25

* Adults.

present work values below 154 and above 161 will be considered abnormal.

Table II contains data from children with tubular nephritis. In the majority two of the serum constituents values—serum protein and serum fixed base—are abnormally low. A low serum protein in tubular nephritis has been found by numerous writers and seems to be a

rather constant finding (7). In our 18 observations the serum protein was low in 16 of the 17 instances in which it was determined. Abnormally low values for total fixed base were found in 14 of the 18 observations and the decrease in the base was in some cases quite marked.

The decrease in the serum base was not accompanied by a proportionate decrease of all the serum acids. The chloride values were all within normal limits, some of them were even at the upper limit of the normal range (96 to 108 cc. of 0.1 N Cl in 100 cc. of serum). The phosphorus was in some cases also rather high (the normal range is taken as being between 2.3 and 3.2 cc. 0.1 N phosphorus in 100 cc. of serum, that is, 4 to 5.5 mg.; the valency of the phosphate mixture in the serum is taken as 1.8, according to Henderson). The bicarbonate value was occasionally somewhat low, but marked acidosis was present only in one patient (the normal limits of the serum bicarbonate in children are, approximately, from 22 to 27 cc. 0.1 N bicarbonate in 100 cc. of serum, that is, 50 to 60 vols. per cent). The decrease in the bicarbonate value was, however, always less than the decrease of the base.

There were, therefore, no very definite changes observed in the amounts of those acids which were directly determined. As to the undetermined acid radicals (chiefly protein and organic acids) an estimation of the base-binding capacity of these acids may be obtained by subtracting the total amount of determined acid from the total fixed base (there is, of course, no free fixed base in the serum). In normal serum, with a fixed base of 157 cc. and a total amount of determined acids of 130 cc. (103 cc. HCl, 25 cc. HCO_3, 2 cc. PO_4) the amount of the undetermined acids would be about 27 cc. Probably the normal variations are quite wide, but there are several values for undetermined acids in Table II which must be considered abnormally low. This decrease in the base-binding capacity of the undetermined acids is probably due to the decrease of the total protein in the serum.

We find, consequently, in most of these cases of tubular nephritis a decrease of the fixed base in the serum and, in the acid radicals, a corresponding decrease of bicarbonate and undetermined acids. Which factor is primary and which secondary is impossible to say. It seems, however, a rather plausible hypothesis, that the loss of protein through

the kidneys is the cause of the low serum protein and that albuminuria, therefore, is the primary change to which all the others are secondary.

In the last column in Table II an attempt has been made to calculate the total amount of electrolytes in the serum.[2] The values are, as is customary, expressed in millimols per kilogram of serum water. The normal value for total electrolytes may be taken as being about 300 mM per kilogram of serum water (8) and is probably a rather constant value (5). In children with acute tubular nephritis there are numerous instances in which the values for total electrolytes are found abnormally low. It seems probable that this decrease in the values for total electrolytes indicates a decrease in the sum of those substances which influence the osmotic pressure of the blood, since urea, the only non-electrolyte of importance in this regard, is not increased in the typical case of tubular nephritis. It must be mentioned in this connection, however, that, according to Atchley, Loeb, Benedict and Palmer (9), there is no depression of the freezing point of the serum in the chronic nephritis with oedema of adults.

In the cases of children with acute glomerular nephritis (Table III) no very definite changes were found in the inorganic constituents of the serum. It is noteworthy that, in a few patients exhibiting alarming symptoms, the findings were quite normal. One patient (case Zois), for instance, had uremia and convulsions at the time when the blood sample was taken; the serum showed a normal content of calcium, total fixed base, chloride and bicarbonate: there was not even an acidosis. In the less severe cases, six of the nine patients had

[2] In order to do this several approximations have been necessary, none of which, however, seriously influences the result of the calculation. As the Fiske method gives the fixed base in terms of cc. 0.1N base it is necessary to know, when transposing the values into millimols, to how large an extent the base consists of divalent calcium and magnesium. (If, for instance, the serum contains 150 cc. monovalent base, Na and K, 5 cc. of Ca and 1 cc. of Mg. then the total fixed base is 156 cc. 0.1 N per 100 cc. of serum, but $150 + 2.5 + 0.5 = 153$ millimols per 1000 cc. of serum.) The magnesium has been neglected in the calculation, as it is present in negligible amount. When the calcium had not been determined, the values of 2 mM per liter has been used. Phosphorus has also been assumed to be present in amounts of 2 mM per liter when it was not determined. The water content of the serum has been calculated from the protein values by means of the formula of Van Slyke, Wu and McLean (8).

a very slightly lowered base value. There were two patients with somewhat low values for chloride and several patients showed a slightly lowered bicarbonate value with a slight acidosis. One patient had a low value for total electrolytes. On the whole, therefore, the slight changes that were present were in the same direction as those found in tubular nephritis, although never as marked as in the latter condition.

In the severe cases there was one (Sylvester), in which the blood

TABLE III

Glomerular nephritis

Values in cubic centimeters of 0.1 N in 100 cc. of serum, except total electrolytes, which are expressed in millimols per kilogram of serum water

CASE	Cl	HCO_3	PO_4	TOTAL ACID	TOTAL BASE	UNDETERMINED ACID	Ca	PROTEIN	NON-PROTEIN NITROGEN	TOTAL ELECTROLYTES
Light cases, oedema slight or absent, normal blood pressure										
Guitarr	94	29	2.2	125	149	24	4.5	7.8		289
Aschodion	95	17	3.0	115	150	35		7.0		277
Aschodion	103	23			156			7.7		303
Golden	103	21	2.8	127	153	26		7.3	40	295
Turner	100	25			156				49	301
Davidoff	99	18			157			6.5	54	291
Davidoff	102	22	2.1	126	152	26		6.8		293
Fondi	103	21	2.6	127	153	26		7.7	52	297
Mack	99	20			152			7.7	47	291
Severe cases, oedema, increased blood pressure, uremic symptoms										
Riordan	103	19	2.6	125	160	35		7.4		302
Zois	101	25			159		4.7			305
McGowan	110	16			166		4.1	6.8		311
Oman	104	23	4.3	131	150	19	5.3		43	294
Sylvester	99	16	4.3	119	145	26	4.2		150	277

picture differed from that of tubular nephritis only in that here there was an increase in the non-protein nitrogen. The other patients did not show any very definite changes with the exception of one case (McGowan), which was the most severe of these cases of glomerular nephritis. In this patient there was a high base, a high chloride and a rather high value for total electrolytes. Possibly this finding indicates

a retention of substances in the blood, caused by deficient functioning of the kidneys.

SUMMARY

In cases of acute glomerular and tubular nephritis in children the total fixed base of the serum has been determined and an attempt has been made to obtain an estimate of the amount of total electrolytes in the serum.

In tubular nephritis the total fixed base is nearly always markedly decreased, and the same is probably true as to the total electrolytes.

In glomerular nephritis there often is a tendency to changes of the same kind as in tubular nephritis, although not so marked as in the latter condition. In one patient with very severe symptoms, however, the base was increased, probably also the total electrolytes. Possibly this finding may be explained as a retention of electrolytes due to impaired kidney function.

REFERENCES

(1) See FISKE, C. H. AND S. S. SOKHEY: *J. Biol. Chem.*, 1925, lxiii, 310.
(2) VAN SLYKE, D. D.: *Ibid.*, 1917, xxx, 347.
(3) BRIGGS, A. P.: *Ibid.*, 1922, lii, 349.
(4) FISKE, C. H.: *Ibid.*, 1922, li, 55.
(5) HAMILTON, BENGT: *Ibid.*, 1925, lxv, 101.
(6) BULGER, H. A., PETERS, J. P., EISENMAN, A. J. AND C. LEE; *J. of Clin. Invest.*, 1926, ii, 213.
(7) LINDER, G. C., LUNDSGAARD, C., D. D. VAN SLYKE, D. D.: *J. Exp. Med.*, 1924, xxxix, 887.
(8) VAN SLYKE, D. D., WU, H., AND F. C. MCLEAN: *J. Biol. Chem.*, 1923, lvi, 765.
(9) ATCHLEY, D. W., LOEB, R. F., BENEDICT, E. M., AND W. W. PALMER: *Arch. Int. Med.*, 1923, xxxi, 611.

TUBULAR NEPHRITIS (NEPHROSIS) IN CHILDREN AND ITS RELATIONSHIP TO OTHER FORMS OF NEPHRITIS[1,2]

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I. CLASSIFICATION OF NEPHRITIS IN CHILDREN

Within recent years there has been much discussion about a form of kidney disturbance to which the term "nephrosis" has been applied (1, 2). This condition is usually distinguished by three clinical features,—the rarity with which red blood cells are found in the urine, the presence of oedema for weeks or months, either continuous or intermittent, and a prolonged course which may end in complete recovery despite frequent exacerbations. Much of the confusion in regard to nephrosis and nephritis is probably due to differences in the definitions of these conditions and it is probable that in every hospital there are cases diagnosed as acute or chronic parenchymatous, exudative, or diffuse nephritis, which are unrecognized instances of nephrosis.

Definition. The classification of renal diseases solely on the basis of clinical symptoms frequently leads to confusion. An etiological differentiation would be the most satisfactory, but in the absence of accurate information in regard to the cause of kidney disease a pathological differentiation is more reliable. Our four autopsies, as well as others which have been reported, indicate that the renal damage in nephrosis is largely tubular. Is then, every case in which the tubules are the principal seat of disease an instance of nephrosis? The usual answer is in the affirmative if the course has been chronic, and in the negative if death has supervened after an illness of a few weeks. Nevertheless, several cases have been described as nephrosis which were fatal within a short time after the onset of symptoms. Perhaps

[1] Received for publication August 9, 1927.

[2] Some of the data contained in this paper were obtained from notes prepared by Dr. John Howland before his death on June 20, 1926.

the only solution of the difficulty will be to reclassify renal disease as it occurs in children. As this procedure has been resorted to in nearly every paper on the subject we naturally hesitate to make another attempt.

There are three main pathological types of nephritis,—*glomerular*, *tubular* and *interstitial*, in which, respectively, the glomeruli, tubules or the connective tissue are the primary seat of the most prominent visible damage. However, the total injury is hardly ever so sharply localized, and in any instance such terms properly define the real situation only in the early stages, or when the damage is very mild; for aside from the fact that functional derangements are not always paralleled by histologically recognizable alterations, severe damage, even if localized to one portion of the histological unit, commonly leads to secondary impairment of the function of the remaining portions. Since the vascular arrangement within the kidney is such that practically all of the blood which supplies the tubules must first pass through the glomerular capillaries, it is obvious that the obliteration of a glomerular tuft will be followed by a disturbance of the function of the dependent tubular epithelium, and if a tubule is destroyed and collapsed, the function of the glomerulus attached to it must be interfered with. Finally, death of the parenchyma may initiate a proliferative and reparative activity of the interstitial tissue, and conversely, if the damage affects primarily the interstitial tissue, the parenchyma may be secondarily compressed or injured in other ways during the inflammatory reaction. In a strict sense, then, a pure form of glomerular, tubular or interstitial nephritis is probably present only during the initial stage; and as the disease progresses, mixed forms occur. However, this pathological classification has a certain degree of usefulness, for when the histologically recognizable damage is most intense in any one of these situations, it is often possible to correlate that damage with a particular clinical course and certain urinary changes (3).

There are at least two clinical features by which the primary pathological changes can usually be diagnosed, namely, the presence or absence of haematuria and oedema. If red blood cells appear in the urine at any time, one can say with assurance that the glomeruli of the kidney are damaged and that these patients are suffering from

glomerular nephritis.[3] At the onset, all of these cases are, of course, acute and one of three events is possible: the patients may recover quickly, or they may die, or their symptoms may be prolonged either intermittently or continuously. If one wishes to subdivide glomerular nephritis, these latter patients can be said to have subacute or chronic glomerular nephritis. However, as this differentiation is made solely upon the duration of the disease and as there is no pathological basis for separating the cases into acute and chronic types, except for the fact that in the latter the involvement of tissues in addition to the glomeruli usually produces a mixed form, and as furthermore there is no means available at present to prognosticate whether a patient, who develops glomerular nephritis, will have a short or long illness, we feel that, so far as children are concerned, the subdivisions of acute and chronic should be discarded for they carry the inference that they indicate two different conditions. The presence or absence of oedema does not appear to bear any relationship to the extent of the glomerular damage or to the associated lesions. One cannot, of course, be certain of the extent to which the tubules and interstitial tissue in the kidneys of these patients may also be affected; in fact, at autopsy in some of these cases the interstitial damage may be very marked, although it is probable that the glomerular changes were the primary lesions.

On the other hand, if there are no red blood cells in the urine at any time or, at the most, only a very few, one cannot say with certainty that there is no glomerular damage or that the patient is suffering from tubular or interstitial nephritis. It is likely, however, that the glomeruli are not implicated to any great extent. If the patient has no oedema at any time during his illness, and the possibility of orthostatic albuminuria has been eliminated, one can often correctly assume that the major part of the damage is *interstitial*,[4] whereas if oedema is present during part or all of the course of the illness and if haematuria

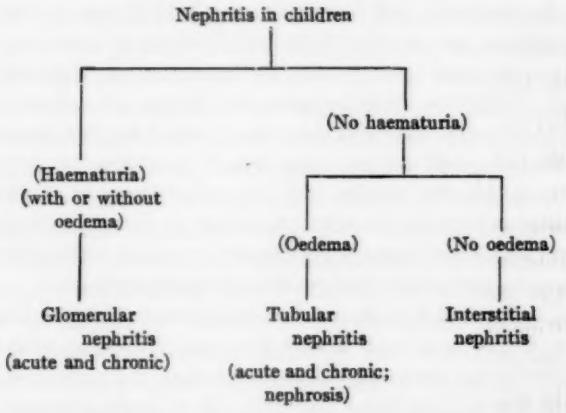
[3] In some very rare cases of interstitial nephritis without glomerular damage, in renal tuberculosis, renal stone and paroxysmal haemo-globinuria, red blood cells may also be found in the urine. In the three latter conditions, the absence of cylindruria and of marked albuminuria simplifies their differentiation from glomerular nephritis.

[4] Pyelitis and pyelonephritis may be excluded by examining the urine for clumps of pus cells.

is persistently absent, one can generally correctly predict that the principal kidney damage is *tubular*. According to the majority of present classifications, if the patient dies or recovers after suffering for a few weeks or months with oedema and albuminuria without haematuria, the diagnosis is *acute tubular nephritis*, while if the course is prolonged, either continuously or intermittently, the patient is said to have *chronic tubular nephritis* or *nephrosis*. This differentiation between acute tubular nephritis and nephrosis is confusing since

TABLE I

*Classification of nephritis in children**



* This diagram is merely a convenient schematic representation of nephritis based upon the primary site of damage. It is to be emphasized that, as the disease progresses, mixed forms occur.

in the case of children there is no valid excuse for the use of two terms. Certainly the length of the illness is not a sound basis, for all of the cases at the onset are acute. It would seem wiser to class all these cases as tubular nephritis because the lesions found at the autopsies on patients who had died after illnesses lasting from 6 weeks to 22 months were practically identical in that the tubules were the chief seat of the damage. The term tubular nephritis is preferable to nephrosis for it emphasizes the primary seat of the damage, but in this paper we have used *nephrosis* as a synonym in order that our

cases may be correlated with those described by others. The use of such a term as parenchymatous nephritis is inadvisable inasmuch as it can be applied equally well to cases in which the glomeruli or the tubules are the principal seat of the damage, since the glomeruli and tubules are both parenchymatous tissue. Exudative and diffuse nephritis are also clinical diagnoses which, in addition to being too indefinite, often cannot be correlated with specific pathological changes. This classification is represented schematically in Table I.

In using haematuria as the basis for differentiating tubular from glomerular nephritis, it must be emphasized that with tubular nephritis a few erythrocytes may be found and that, with glomerular nephritis of long duration, blood may be absent from the urine for days and even weeks. It is, therefore, only after prolonged observation that one may properly decide that haematuria is absent. It is really the clinical course that must determine the diagnosis; when there are persistent albuminuria and cylindruria with oedema, a blood pressure which is rarely much above normal, a urine often concentrated and free from blood, little or no evidence of nitrogenous retention in the blood and no striking deterioration in the patient's general condition despite albuminuria and oedema that persist for weeks and months it is justifiable to make a diagnosis of tubular nephritis.

Something must be said regarding the blood pressure in children, for an absence of increase is claimed to be of great assistance in differentiating tubular nephritis (nephrosis) from the glomerular or interstitial form. A child who is suffering from glomerular or interstitial nephritis develops an increased blood pressure with no such regularity as does an adult. We have seen children with progressive and eventually fatal glomerular or interstitial nephritis with but little increase, in blood pressure; in fact at times, even for weeks, in such patients it may be slightly subnormal. A blood pressure determination is, therefore, not a reliable observation by which to exclude glomerular or interstitial nephritis in children.

II. TUBULAR NEPHRITIS (NEPHROSIS)

Symptoms and signs. (See Table II.) These patients are generally brought to the hospital because of the presence of *oedema*. This may develop without any preceding infection, or, may appear shortly after

an acute infection, usually of the upper respiratory tract. Ordinarily, the onset is insidious, occasionally very rapid; at times it is steadily progressive, at others presenting marked daily fluctuations. In 61 per cent of our patients the onset was gradual and in 39 per cent it was sudden. Nausea and vomiting often usher in the attack which may be accompanied in some cases by suppression of urine. Extreme degrees of oedema may be seen, the eyes being closed, the legs swollen, marked ascites being present and, in males, the scrotum distended with fluid. In spite of the oedema, unless this be extreme, the patients

TABLE II

Clinical and laboratory data in 26 patients suffering from tubular nephritis (nephrosis)

	AGE AT ONSET	DURATION (MONTHS)				BLOOD PRESSURE ON ADMISSION		URINE ANALYSES				BLOOD ANALYSES		
		All patients	Patients who are entirely well	Patients who died	Patients who are improved but who still have albuminuria	Systolic (mm.)	Diastolic (mm.)	Quantity in 24 hours (cc.)	Specific gravity	Albumin, grams per liter	Phthalein excretion (per cent)	White blood cell count on admission	Haemoglobin (Sahli) (per cent)	Non-protein nitrogen (mgm. per 100 cc.)
Average.....	5 years	11	8	17	9	110	75	450	1018	8	57	10,600	69	34.5
Minimum....	8 months	1	2	1½	1	100	45	31	1003	3	20	6,000	52	23
Maximum....	11 years	72	36	36	72	150	110	2,480	1040	18	95	32,600	95	85

are often comparatively comfortable and have a fair appetite. This disproportion between the degree of oedema and the constitutional symptoms is in contrast to the prostration often exhibited by oedematous patients suffering from glomerular nephritis. The tendency to the collection of fluid in the serous cavities is quite different with different children. In some, hydrothorax is present and recurs promptly after thoracentesis, the same being true for ascites, while with others the fluid seems to be confined almost entirely to the subcutaneous tissues. In some children the oedema may rapidly change its location; on one day the face may be markedly oedematous and on the

next quite normal in appearance even though the weight remains the same. To the variations in oedema are to be ascribed the striking fluctuations in weight. The weight of one child, 18 months old, without any change in diet or medication fell from 33 to 22 pounds and rose again to 37 pounds in a short time. The oedema may persist for several days and then gradually disappear so that at the end of two to three weeks the patient may be quite normal in appearance. In other cases, the accumulated fluid may subside in two or three days, the albuminuria markedly diminish and occasionally the urine become free from albumin. In many instances, however, the oedema does not disappear entirely even after several weeks and an appreciable amount of albumin remains in the urine. Whether the oedema entirely disappears or not, there are frequent periods, weeks or months apart in which exacerbations occur. Fifty per cent of our patients had more than one attack. One of these children (patient no. 8) has had at least thirteen such periods during the past five years and has spent a total of 30 months of this time in the hospital. At times she has remained free from oedema for as long as twelve consecutive months although albumin has never entirely disappeared from her urine; at other times, she has been more or less oedematous for periods of one to four months. Injections of foreign protein, such as therapeutic sera, usually cause a rapid increase in oedema; the routine intradermal tuberculin test seemed to have the same effect in two of our patients. A skin rash, consisting of irregular red macules and occasionally papules, has been noted on the face, abdomen and back of several of our patients during their oedematous periods.

The *duration* of the disease in the six children who have apparently completely recovered has varied from 2 to 36 months, the average being 8 months. Among the six who died, the average length of the illness was 17 months and the extremes were 6 weeks and 36 months, while among those who are improved in health but not free from albuminuria the average duration was 9 months and the minimum and maximum 1 and 72 months, respectively (Table II).

Urine. The volume of the urine varies, particularly with the development and subsidence of the oedema (Chart 1). It may be diminished or increased to a striking degree. The volume of urine in 24 hours varied from 31 to 2480 cc., the average being 450 cc. (Table

II). At the height of the oedema or when the child is free from it, the urinary volume varies with the fluid intake. The kidneys are, however, capable of excreting a very concentrated urine. Fixation of specific gravity is not found. It has varied from 1003 to 1040 with an average in all patients of 1018 (Table II). Another variable factor is the amount of albumin in the urine which may be only a trace or may cause the urine on boiling to become nearly solid. The amount of albumin has varied from 3 to 18 grams per liter, the average being 8 (Table II).

Despite the presence of a few white blood cells and of hyaline and

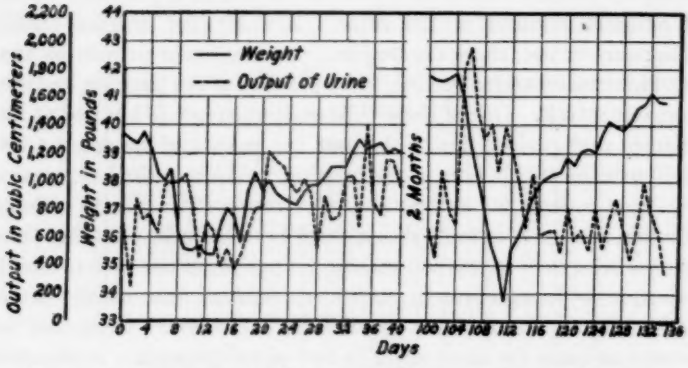


CHART 1. COMPARISON OF OUTPUT OF URINE AND LOSS OF WEIGHT (PATIENT NO. 8)

granular casts in the urine of all of our patients, at times in enormous numbers, repeated examinations in our 26 patients failed, except in two instances, to disclose the presence of blood by the guaiac or other chemical tests. A few red cells were seen with the microscope in four of our cases, probably as a result of the secondary involvement of a few of the glomeruli. Macroscopic blood was found in the urine of only one of our patients; he was suffering from epidemic influenza at the time. Doubly refractile lipoid bodies, the presence of which has been considered so significant for the diagnosis of nephrosis in adults, have been found at times in large quantities. At other times repeated search is necessary to disclose them and they may also be found in

small numbers in glomerular and interstitial nephrosis of long duration. Their presence in great numbers may assist somewhat in the diagnosis but their absence does not exclude the possibility of nephrosis. Clausen (4) has described a substance in the urine of these patients which reduces surface tension.

In striking contrast to the oedema, which may equal or exceed that found in any other condition, the functional capacity of the kidneys, as measured by the excretion of phenolsulphonephthalein, is usually excellent. An excretion greater than the normal may even be found. The average excretion of 'phthalein at the end of two hours was 57 per cent with extremes of 20 and 95 per cent (Table II).

TABLE III

*The inorganic constituents of the blood in tubular nephritis (nephrosis) (expressed in millimols)**

CASE NUMBER	PER CENT OF CORPUSCLES	CHLORIDES (SERUM)	CALCIUM (SERUM)	SODIUM			POTASSIUM			TOTAL OF SODIUM PLUS POTASSIUM IN CORPUSCLES
				Serum	Whole blood	Corpuscles	Serum	Whole blood	Corpuscles	
6	45	101.41	2.05†	126.96	77.83	18.26	6.45	45.01	88.49	106.75
21	34.5	78.31		126.96	92.17	26.52	3.48	33.22	89.77	116.29
Normal control	40	101.97	2.50	145.65	84.78	0	4.99	46.29	109.46	109.46

* We are indebted to Dr. S. G. Ross and Dr. F. F. Tisdall for these chemical studies.
† None in corpuscles.

Blood. When the oedema is increasing rapidly, owing to water retention, there must obviously occur also a retention of salt, and during these periods the chlorides and cholesterol of the blood are increased. In Table III is given a study of the inorganic constituents of the blood of two patients whose oedema was practically stationary. The only marked deviations from the normal are the increase in the amount of sodium in the corpuscles and the decrease in their potassium content; the total amount of base (sodium plus potassium) in the corpuscles differs but little from the normal. We have not found patients in the stage of high blood concentration that Volhard and Fahr (1) have described as typical of the period when the vessel walls are permeable to the fluid portions of the blood. The haemoglobin

content was over 85 per cent in only two patients, the average being 69 per cent (Sahli) with minimal and maximal values of 52 and 95 per cent, respectively. Our refractometric observations have shown the serum protein to be somewhat reduced, which perhaps may be interpreted as evidence of a blood dilution. This, however, is not particularly characteristic of tubular nephritis. It is found in other forms of nephritis with oedema. The normal albumin-globulin ratio of the blood is reversed, because a large proportion of the protein which is lost in the urine is albumin. The low plasma proteins and the invertion of the albumin-globulin ratio of the plasma are almost the exact reciprocals of those of the urine (5) (Table IV).

The ability of the kidney to excrete the end-products of protein metabolism is usually unimpaired. We have rarely found an increase

TABLE IV

Plasma and urine proteins in tubular nephritis (nephrosis) (5)

CASE NUMBER	DATE	PLASMA		URINE	
		Total protein (grams per cent)	Albumin-globulin ratio	Total protein (grams per cent)	Albumin-globulin ratio
6	April 13, 1926	4.26	17/83		
7	June 1, 1925	3.97	29/71	1.4	100/trace
18	March 22, 1926	3.88	18/82	1.3	80/20
	April 16, 1926	5.40	17/83	0.6	75/25

in the amount of urea and non-protein-nitrogen in the blood. The average amount of non-protein-nitrogen in the blood was 34.5 mgm. per 100 cc. with extremes of 23 and 85. This last value was the only one in our patients while in this hospital which was much above normal limits. The patient was discharged as improved but still had albuminuria when he was last seen. In one other patient, who died three years later in another hospital (patient no. 16), the non-protein-nitrogen of the blood, at the time of death, was 121 mgm. per 100 cc. No autopsy was performed, but it is probable that he had marked glomerular and interstitial damage in addition to his original tubular nephritis. Shortly before his death, there were red blood cells in his urine, his 'phthalein excretion was 5 per cent and his blood pressure 176/126.

The *blood pressure* of four of our patients was elevated, but the rise was merely transient. The average blood pressure in this series was 110/75 with extremes of 100/45 and 150/110 (Table II).

The *age* of the patients at the time of the onset of the disease has varied from 8 months to 11 years with an average of 5 years (Table II). Tubular nephritis is apparently much more common in white boys than in colored boys or in white or colored girls (Table V). Twenty-five per cent of our patients were of Slav or Czech parentage,

TABLE V

Sex and color distribution

SEX AND COLOR	NUMBER OF PATIENTS	CASE DISTRIBUTION (IN PER CENT)	SEX AND COLOR DISTRIBUTION OF HARRIET LANE PATIENTS (1912-1922 INCL.; 32,892 PATIENTS) (IN PER CENT)
Male, white	16	61.5	38.8
Female, white	6	23.1	35.0
Male, colored	1	3.8	13.8
Female, colored	3	11.6	12.4
White (both male and female)	22	84.6	73.7
Colored (both male and female)	4	15.4	26.3
Male (both white and colored)	17	65.4	52.6
Female (both white and colored)	9	34.6	47.4
Total	26	100.0	100.0

a much larger proportion than one would expect in Baltimore, though this may have been a coincidence.

Uremia has not been observed, although two of our patients had convulsions at irregular intervals for a period of several days. These convulsions were probably not uremic, as they seemed to bear no relation to the presence of oedema, nor could they be explained on the basis of any changes in the constituents of the blood.

Complications. The frequency of pneumococcus peritonitis among patients suffering from tubular nephritis is very interesting but as yet is not understood. In 6 of our 26 patients this infection has occurred during a period of oedema. Four of these patients died, two during

the height of the pneumococcus peritonitis and the other two 9 and 12 months, respectively, after complete recovery from their peritoneal infections. In these four children the type of pneumococcus was determined—Type II in two instances and Type I and Type IV once each. In one patient, the oedema disappeared completely within ten days after the development and drainage of the pneumococcus peritonitis, only to reappear after this infection had receded (Chart 2).

Mortality. Of the 20 patients of whom we have present records, six (30 per cent) are dead. If one of these patients is excluded because he has been observed less than one year the corrected case fatality would be 32 per cent. This figure is somewhat lower than that of

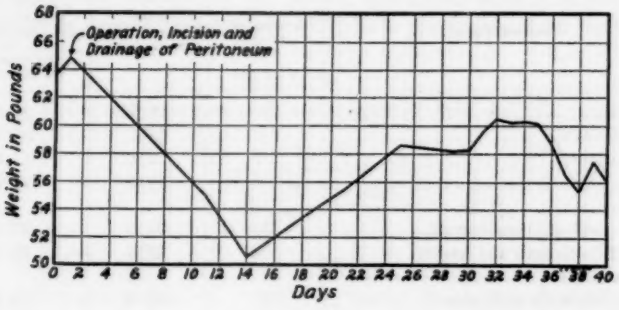


CHART 2. LOSS OF OEDEMA DURING PNEUMOCOCCUS PERITONITIS (PATIENT NO. 23)

Clausen's series (4) in which 43 per cent died. Autopsies were obtained on all of the four patients who died in this hospital and the direct cause of death in two of them was bronchopneumonia and in the other two pneumococcus peritonitis—a fact that would emphasize the susceptibility of these children to infections. One of the two latter also developed erysipelas and pneumococcus (Type II) septicemia, pneumonia and pleurisy. In spite of this rather high mortality, complete recovery can apparently occur, for 6 of our patients are entirely well without albuminuria, and 5 of these have remained so for over seven years.

Etiology. Tuberculosis and syphilis have been claimed to be frequent etiological factors, but they are probably not responsible for

this condition, since among the 26 patients whom we have seen, only one had syphilis and one tuberculosis. Three other children had positive reactions to the intradermal injection of 0.1 mgm. of tuberculin (O.T.), but they had no clinical manifestations of tuberculosis. None had scarlet fever or diphtheria recently preceding the onset of the tubular nephritis. One child had never been ill before. Clausen (4) and Marriott (6) have reported that an infection of the nasal accessory sinuses with a staphylococcus is practically always the cause of parenchymatous nephritis which is their synonym for tubular nephritis (nephrosis). We have seen one case for which this statement may have held, for the symptoms were somewhat alleviated after the antra were irrigated (Chart 11), and Aldrich (7) has reported several such cases. However, careful and repeated examinations of the nasal accessory sinuses have demonstrated that among our 26 patients only 5 had clouding of one or more sinuses. Irrigation of the sinuses was performed on 3 of these patients but no pus was obtained. In two of these 3, no diminution in the oedema followed irrigation (Charts 12 and 13). The two patients whose sinuses were clouded but not irrigated lost their oedema as rapidly as the other children. Bessau (8) in his series of cases of tubular nephritis in Leipzig was unable to demonstrate any infection of the accessory nasal sinuses. Perhaps the frequency of sinus infection varies in different localities. It must also be borne in mind that these patients have very little resistance to pneumococcal infections (4, 9), as is witnessed by the frequency of pneumococcus peritonitis among them and also by the fact that the direct cause of death in the fatal cases is usually an infection. It is possible that the infections of the nasal sinuses described by others in cases of nephrosis may have been coincidental but not causal. That 74 per cent of our patients were afebrile and that the average white blood cell count on admission was normal (Table II) furnish additional evidence against the supposition that an infection is the cause of tubular nephritis. It is, of course, possible that more energetic examination of the sinuses by diagnostic puncture may have revealed infections which were not demonstrated by X-ray, transillumination and the rhino-pharyngoscope, but a comparison of the mortality of our series with that in which diagnostic punctures were done (4) would seem to indicate that this procedure is not without danger.

If tubular nephritis (nephrosis) is due to or associated with an infected sinus, perhaps it is safer not to search for it too vigorously, for these patients apparently have very little resistance to infections such as may follow operative procedures on the nose and throat. Only 6 of our 26 patients had suffered from an infection immediately preceding the onset of tubular nephritis—two of these had had tonsillitis, 3 rhino-pharyngitis and one osteomyelitis. Unless one is to consider as an etiological agent an illness which antedated the onset by several years, one must conclude that in our series there has been no apparent causative factor.

As a pure speculation in regard to the etiology of nephritis, one could draw an interesting analogy from the effect of mercury and uranium on the kidneys. If these chemicals are given to certain animals in small amounts over a long time, the damage to the kidneys is predominately tubular, whereas, if large doses are given, the glomeruli are markedly implicated. Could it be possible that the same "toxin" causes both tubular and glomerular nephritis, and that if the rate of absorption is slow, tubular damage predominates, and that, if rapid, the glomerular lesions are prominent?

Lesions. The first of the four autopsies[5] was on patient no. 25, a white boy, six years old, who had been ill for only six weeks and who had pneumococcus (Type II) peritonitis and erysipelas for five days prior to death. The kidneys were enlarged, weighing 100 grams each and measuring 11 x 4 x 3 cm. They were rather "rubbery" in consistence. The capsules were not adherent and the surfaces were smooth. The color was a pale yellow grey and there were numerous flecks of bright yellow and a few punctate haemorrhages. On section, the cortices were thick, measuring 1 cm., the striae were indistinct, the color was grey tinged with yellow, and many bright yellow flecks were scattered throughout the cortices. Haemorrhages were difficult to find on the cut surface. The medullae were pink. The pelves and ureters were normal. Microscopically, there were hyaline thrombi in many of the glomeruli and also fresh adhesions between some of the tufts and Bowman's capsules. No scarring of the glomerular tufts or of the pericapsular tissue was found. Albumin was present in the cap-

[5] We are indebted to Dr. Arnold R. Rich for the description of these four autopsies.

sular spaces, but blood cells in this situation were rare. However, numerous tubules contained an abundance of red blood cells, hyaline casts, albumin and an occasional leucocyte. The tubular epithelium in most places was swollen and granular. This was especially noticeable in the convoluted tubules. In some cells vacuoles were found which undoubtedly represented spaces occupied formerly by a part of the large quantity of fat which was stainable in frozen sections with Sudan III. The fat droplets were especially numerous near the basal portions of the epithelial cells. In contrast to the swollen cells lining most of the tubules there were other tubules which were dilated and lined with very flat atrophic epithelium. These tubules had the appearance of those seen in patients who have died of nephritis of long duration, and most probably represented the end-result of dilatation following obstruction of the tubular lumen by a cast. The epithelium was so atrophic that it could hardly have been of much functional use. Hyaline casts filled many of these tubules. The interstitial tissue, blood vessels and pelves were normal.

The second autopsy was on patient no. 17, a white boy, aged five years, whose illness had lasted six months. He had pneumococcus (Type IV) peritonitis for six weeks prior to death. The kidneys were greatly enlarged and pasty in consistence. The capsules were not adherent and the surfaces were smooth. The cortices were yellowish-grey in color, flecked with many distinctly yellow opacities. Microscopically, all the glomeruli throughout the sections were entirely normal in appearance save for the presence of albuminous material within the capsular spaces. The interstitial tissue was likewise normal. The tubules, however, were everywhere dilated, and were often lined with greatly swollen and granular epithelial cells which contained large amounts of doubly refractile lipoid. Here and there small masses of necrotic coagulated tubular epithelium were found. There were hyaline casts and much albuminous material within the tubules and a few of them contained fresh red blood cells. In this case the anatomically recognizable damage was confined sharply to the tubular epithelium, although the presence of blood in the tubules suggested glomerular damage as well.

The third autopsy was on patient no. 15, a white girl, four years old, who had had three exacerbations of her symptoms, a total illness of

twelve months, and who had had pneumococcus (Type I) peritonitis from which she had completely recovered nine months before her death. The consistence of the kidneys was rather pasty, the surface beneath the adherent capsule was finely granular, and yellowish flecks and streaks were prominent in the cortex on section. Microscopically, there were numerous rather isolated scarred areas containing altered glomeruli and collapsed tubules, some of the latter being calcified. Small calcified masses were occasionally seen in the interstitial tissue just outside tubules. Most of the glomeruli outside the scarred areas were quite normal in appearance, but in and near the scars there were glomeruli with tufts partly or completely replaced by connective tissue, and with capsular spaces in various stages of obliteration by a concentric growth of connective-tissue cells. Throughout the kidney the tubular epithelium contained droplets of doubly refractile lipoid. Within the capsular spaces and tubules there was albuminous material but no blood cells. The blood vessels were normal.

The fourth autopsy was on patient no. 23, a colored boy, eleven years old, who had been ill for 22 months with four exacerbations of his symptoms, and who had had pneumococcus (Type II) peritonitis from which he had recovered one year before his death. The kidneys were large weighing about 200 gms. each and measuring 12 x 7.5 x 4 cm. They were somewhat pasty. The capsules stripped easily leaving smooth surfaces, of a strikingly pale grey color mottled with bright yellow flecks. On section the cortices were thick, measuring nearly 1 cm. The regular striae were swollen, the color of the cortices was grey with a waxy translucency, and flecking this background were many points of yellow representing accumulations of fat in the epithelial cells. The medullae were pinkish grey. There were no haemorrhages. The pelves and ureters were normal. Microscopically, the kidney showed advanced autolysis (the autopsy was done two days after death) which interfered with the proper evaluation of alterations in the appearance of the tissue. The glomeruli had a normal appearance. There was nothing which indicated that the cells composing them were the site of any sort of destructive process—no capillary thrombi, no adhesions, no scarring, no alteration of Bowman's capsule. That the glomerular tufts were functioning abnormally, however, was obvious from the presence of albumin within the capsu-

lar spaces. There was no evidence of any escape of blood cells from the tuft capillaries. The tubular epithelium was so autolyzed that any description of the appearance of these cells would be of no value, except that many of them contained large vacuoles which were in keeping with the large globules of fat which were stainable with Sudan III in frozen sections. Fat stains also showed a large number of minute droplets of fat, widely distributed throughout the extent of the tubules, though most abundant perhaps in the proximal convoluted tubules and in the descending portions of Henle's loops. The fat droplets tended especially to lie near the basement membrane of the epithelial cells. There were several areas in the sections in which there were interstitial accumulations of mononuclear cells of the plasma cell, lymphocyte and clasmatocyte variety. A few polymorphonuclear leucocytes were scattered among the mononuclear cells. Occasionally these cells had found their way into adjacent tubules and were conglomerated in the lumen. The polymorphonuclears predominated in this invasion. Such areas, however, were small and rare. The interstitial tissue appeared oedematous in one section, the tubules being widely separated, but this was not so conspicuous in the sections from the other kidney. There were isolated obliterated glomeruli associated with the minute scars representing the collapse of dependent tubules, but they were found no more frequently than in the average "normal" kidney. The blood vessels and pelves were normal.

To judge from the autopsies on these four patients, the damage to the kidneys in tubular nephritis (nephrosis) is predominately tubular, in contradistinction to the changes in glomerular and interstitial nephritis in which the glomeruli and interstitial tissue are implicated to a much greater extent. Of course, as was pointed out earlier, it would be erroneous to speak of a pure tubular nephritis and a pure glomerular or interstitial nephritis, for various intermediate stages occur; even if the primary damage be localized to the tubules, secondary derangement of the glomeruli may follow and vice versa. The paucity of the glomerular lesions in tubular nephritis explains the characteristic absence of the red blood cells in the urine of these patients.

In all of these autopsies, although the tubular lesions were the predominating feature, the glomeruli were also found to be somewhat damaged, particularly in the first autopsy (patient no. 25) in which

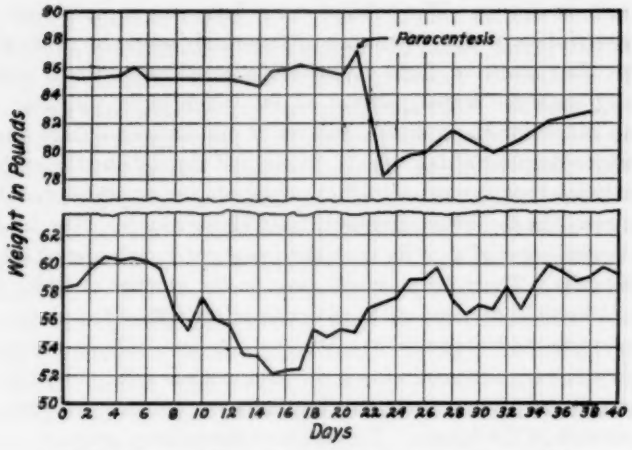


CHART 3. SALT-FREE DIET (PATIENTS NOS. 8 AND 23)

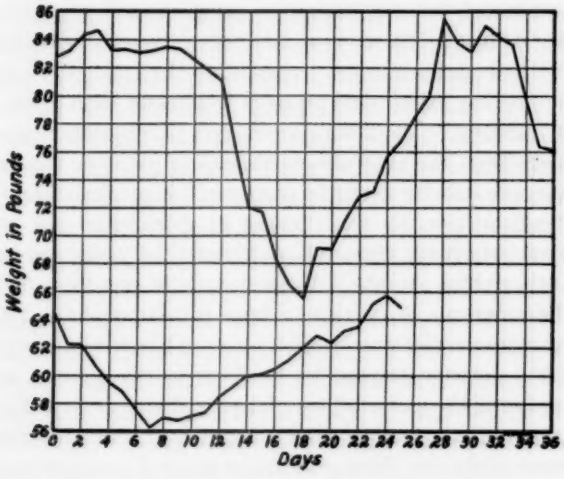


CHART 4. NORMAL DIET (PATIENTS NOS. 8 AND 23)

there was obviously a fairly fresh glomerular nephritis. After repeated search, three red blood cells were found in this boy's urine on one occasion and the guaiac test in this specimen was positive, but erythrocytes were absent and the guaiac test negative in numerous

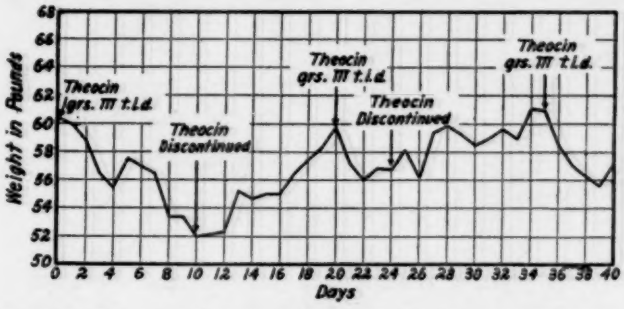


CHART 5. LOSS OF OEDEMA DURING TREATMENT WITH THEOCIN (PATIENT NO. 23)

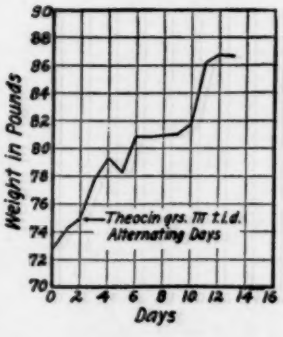


CHART 6. INCREASE OF OEDEMA DURING TREATMENT WITH THEOCIN (PATIENT NO. 8)

other examinations. No red blood cells were seen in the urine of the other three patients and the guaiac test was repeatedly negative.

The interstitial lesions were insignificant except in the fourth autopsy (patient no. 23), in which the small, rare areas of interstitial accumulations of mononuclear cells had the appearance of an interstitial nephritis.

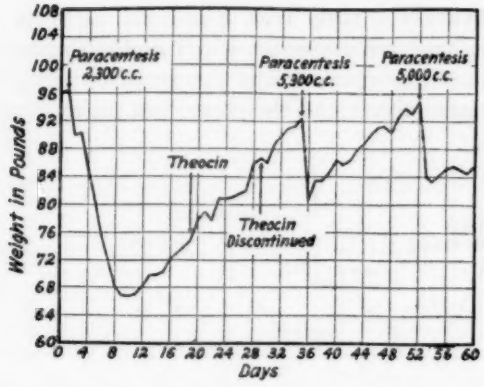


CHART 7. LOSS OF OEDEMA FOLLOWING ABDOMINAL PARACENTESIS
(PATIENT NO. 8)

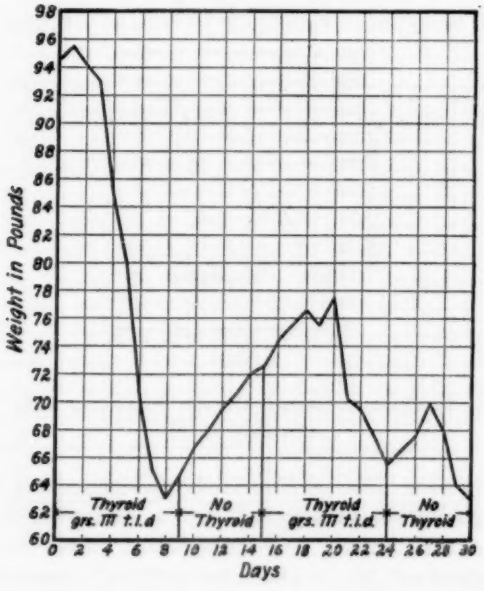


CHART 8. LOSS OF OEDEMA DURING TREATMENT WITH THYROID EXTRACT.
(PATIENT NO. 8)

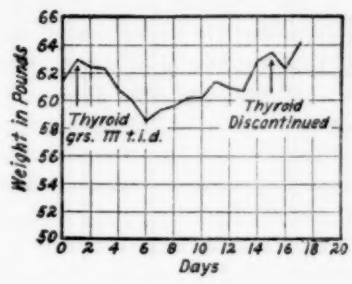


CHART 9

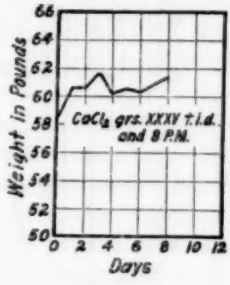


CHART 10

CHART 9. NO EFFECT FROM TREATMENT WITH THYROID EXTRACT (PATIENT NO. 23)

CHART 10. NO EFFECT FROM TREATMENT WITH CALCIUM CHLORIDE (PATIENT NO. 23)

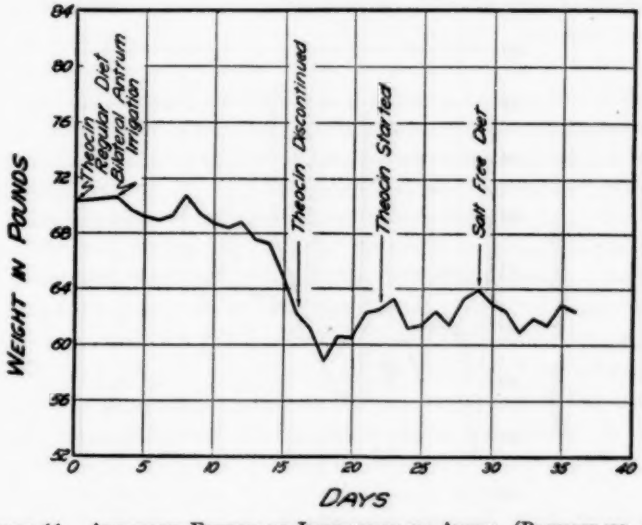


CHART 11. APPARENT EFFECT OF IRRIGATION OF ANTRA (PATIENT NO. 23)

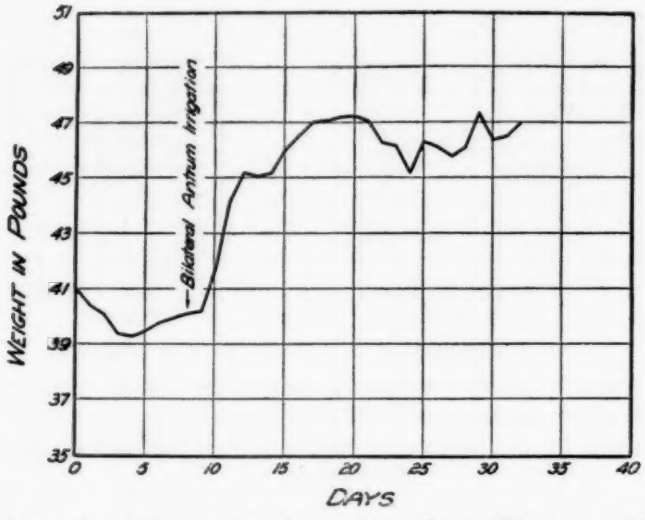


CHART 12. NO EFFECT FROM IRRIGATION OF ANTRA (PATIENT NO. 11)

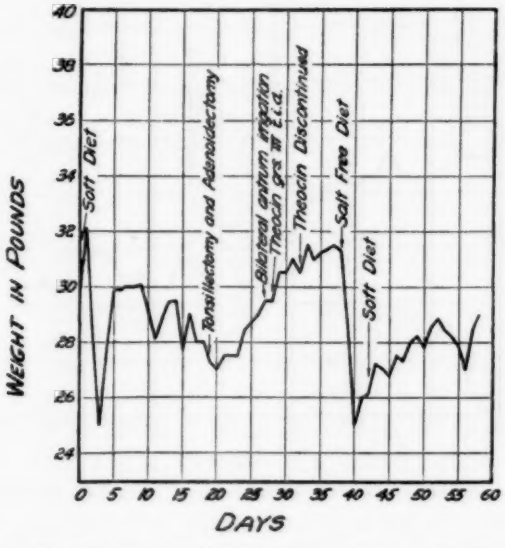


CHART 13. NO EFFECT FROM IRRIGATION OF ANTRA. (PATIENT NO. 26)

Treatment. Theoretically a salt-free, low protein diet might seem to be indicated. As a matter of fact in some of our patients such diets did not bring about a decrease of the oedema. (Chart 3). In the instances in which it was given, a high protein diet had no effect. Other patients have lost and then reaccumulated their oedema while taking a normal diet (Chart 4 and 13), so that it would seem that the dietary treatment of tubular nephritis is usually ineffectual. Diuretics, such as theobromine sodium salicylate and theocin, will often cause a loss of oedema, but the fluid usually reaccumulates as soon as the drug is discontinued (Chart 5). On the other hand, some patients are unaffected by theocin, and their oedema may even increase during its administration (Chart 6). If the ascites is excessive, paracentesis is, of course, indicated; and occasionally a rapid loss of oedema will follow this procedure (Chart 3 and 7). Thyroid extract has been recommended for these patients (10). In some children the oedema will disappear during treatment with thyroid extract (Chart 8), but the loss is only transient. In other patients, no effect can be noted (Chart 9). The administration of calcium chloride (Chart 10) and of intestinal purges did not produce any lasting benefit. An examination of the nasal accessory sinuses by the usual methods is, of course, advisable and, if infected, they should be drained. In one of our patients this procedure was apparently beneficial although the disappearance of oedema may have been due to the theocin which was administered coincidently (Chart 11). In other children no diminution of oedema followed irrigation of clouded antra (Charts 12 and 13). We feel that children suffering from tubular nephritis (nephrosis) should be kept in bed and given a normal light diet during their periods of oedema but that they should be allowed their usual activity at other times even though the albuminuria persists. Basch (11) has recently stated that the renal function and diuresis are increased and the albuminuria improved if, after the fourth to the fourteenth day of the disease oedematous patients are allowed to be ambulant during the day, except for a rest period of two hours. We are now investigating this practice.

Summary. During the past fifteen years among 53,965 ill children in the Harriet Lane Home, twenty-six have been diagnosed as having tubular nephritis (nephrosis). Six of the twenty patients regarding whom

we have definite information are well, and five of these have been so for more than seven years. Six of the twenty are dead. In eight, the disease is still active, although it cannot be said that they may not eventually recover. Unless our experience during the last ten years has been unique, it would seem that at least a fair proportion of children with tubular nephritis recover. However, very few reports of complete recovery are to be found in the literature and until the last few years there was no indication that the condition had been recognized by pediatricians. We cannot ascribe the recovery to any particular form of treatment; improvement and relapses may occur without any change in therapy. These cases indicate (1), that tubular nephritis (nephrosis) is a clinical as well as a pathological entity, which can usually be recognized by its clinical course, (2), that the etiology is as yet unknown, (3) that the prognosis is better than other reports would seem to indicate, although it must be emphasized that the duration may be long and that several years may elapse before the patient may be permanently free from oedema and albuminuria and (4) that at autopsy the tubules of the kidney are more markedly implicated than the glomeruli and interstitial tissue, although they, too, are damaged. We believe that the term, *tubular nephritis*, is preferable to *nephrosis* and that the presence or absence of haematuria and oedema is the most satisfactory criterion at present for the classification of nephritis in children into its three principal anatomical varieties—glomerular, tubular and interstitial.

REFERENCES

(1) Volhard, F., and Fahr, T. L.: Die Brightsche Nierenkrankheit. Berlin, 1914.

(2) Munk, F.: Pathologie und Klinik der Nephrosen, Nephrite und Schrumpfnieren. Berlin, 1918, Urban u. Schwarzenberg. 376 pp.

(3) Holt, L. E., and Howland, J.: The diseases of infancy and childhood. 9th ed., New York and London, 1926. D. Appleton and Co. 1018 pp. (The section on nephrosis in this book is based on the data in this paper.)

(4) Clausen, S. W.: Parenchymatous nephritis. *Am. J. Dis. Child.*, 1924, **29**, 581–586, 587–593, 594–602.

(5) Perlzweig, W. A., Allen, W. M., and Salinger, R.: The relation of the concentration of proteins in the plasma and urine in various conditions. *Johns Hopkins Hospital Bull.* (in press).

(6) MARRIOTT, McK.: Nephritis; newer viewpoints concerning its nature and treatment. *Northwest. Med.*, 1925, **24**, 472–479; *J. Michigan M. Soc.*, 1925, **24**, 650–653.

(7) ALDRICH, C. A.: The treatment of nephrosis. *Am. J. Dis. Child.*, 1926, **32**, 163–182.

(8) BESSAU, G.: Personal communication.

(9) ELWYN, H.: The pathogenesis of lipoid nephrosis. *Arch. Int. Med.*, 1926, **38**, 346–359.

(10) EPPINGER, H.: Zur Pathologie und Therapie des menschlichen Oedems. Berlin, 1917.

EPSTEIN, A. A.: Thyroid therapy and thyroid tolerance in chronic nephrosis. *J. Am. M. Ass.*, 1926, **87**, 913–918.

(11) BASCH, F.: Klinisches und Experimentelles zur Behandlung nierenkranker Kinder. *Ztschr. f. Kinderh.*, 1926, **42**, 589–605.

APPENDIX

The following twenty-six patients (with their Harriet Lane Home history numbers) are included in this study:

1—H.A.M. (4904)
2—C.C. (5617)
3—O.W. (12113)
4—E.L. (17724)
5—W.B.L. (18154)
6—P.E. (19022)
7—C.C. (19117)
8—C.S. (22936)
9—H.E.P. (23673)
10—M.K. (26476)
11—S.D. (27916)
12—D.S. (29019)
13—M.D. (30354)
14—E.M. (30397)
15—M.C. (31422)
16—W.B. (31789)
17—J.H. (39400)
18—M.M. (39817)
19—F.M. (40104)
20—J.O'D. (41490)
21—H.C. (45326)
22—C.O. (46558)
23—E.W. (47062)
24—D.B. (50083)
25—T.W. (51288)
26—H.S. (52264)

BULLOUS IMPETIGO COMPLICATING VARICELLA

Report of a Case

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Chicken-pox is one of the mildest of the exanthematous diseases, and its very rare fatalities are due not to the disease itself but to complications. The more frequent complications are those arising from infection of the skin about the chicken-pox lesions, *e.g.*, impetigo, erysipelas, boils, subcutaneous abscesses, and gangrene. The most common of these complications is impetigo. Speaking of its incidence, Schamberg (1) says: "Most well-marked cases of chicken-pox show some lesions which become the seat of impetigo." It may occur about one or several vesicles, manifesting itself by a slight delay in healing or by a peripheral extension of the original lesion, thus definitely establishing the existence of a second distinct disease. To designate such peripheral extension Welch and Schamberg (2) adopted the name *impetigo varicellosa*, and stated that it was due to infection of the lesion with the pyogenic organisms commonly found upon the skin. A few cases of chicken-pox complicated by a bullous eruption similar to that of pemphigus neonatorum or impetigo bullosa have also been described. Formerly, such cases were spoken of as *varicella bullosa*. At present, however, the term has been discarded, inasmuch as the bullous lesions are now recognized as being due to a complicating infection rather than to the virus of varicella. In this paper a case of chicken-pox is reported in which the bullous lesions were definitely shown to be due to a complicating staphylococcic infection.

REPORT OF CASE

R. B., male, aged 13 months. Admitted February 7, 1927, with a diagnosis of chicken-pox. No *family history* obtainable.

Past history. At 2 months of age the child had been admitted because of malnutrition to one of the hospitals of the city, where he had remained until the

onset of the present illness. At 4 months of age he had had an uncomplicated attack of measles. He had had no other diseases.

Physical examination on admission. Temperature 100.2°F., pulse 124, respirations 24. The child is undernourished and undersized. He does not appear acutely ill. He lies in bed quietly except for an occasional rolling of the head from side to side. The negative findings are omitted.

Glands: The posterior cervical, the axillary, and the inguinal lymph glands are large and firm. There is a gray purulent odorless discharge from the left ear. The tonsils are large but not inflamed.

Skin: The skin is moist and warm. An eruption is evident chiefly over the chest, abdomen, and back, involving to a lesser extent the face, scalp, and upper portions of the extremities. This eruption consists of discrete lesions, most of which are red papules, 2 to 4 mm. in diameter. There are a few clear vesicles, 3 to 5 mm. in diameter, surrounded by a red areola. Some of the vesicles are umbilicated, others have been ruptured, but none as yet have become incrusted. There is in the region of the right groin an area of exfoliated skin, 2 cm. in diameter, the edge of which shows the thin, peeling epidermis. The distribution and the character of the lesions, with the exception of the one just described, are typical of chicken-pox.

Summary of positive findings. Physical examination of the patient on admission reveals an undernourished child with a discharge from the left ear, a mild coryza, an enlargement of the posterior cervical, axillary, and inguinal lymph glands, an eruption characteristic of chicken-pox, and an area of superficial exfoliation of the epidermis surrounding one of the varicella lesions.

Laboratory examination. Cultures from the nasal discharge on Loeffler's medium revealed no diphtheria bacilli. Cultures on blood-agar plates from the discharge from the left ear gave a hemolytic *Staphylococcus aureus.*

Course in the hospital. Feb. 8. There were many fresh varicella lesions on the trunk and face. The proportion of clear vesicles to papules had increased. More of the lesions had become umbilicated and crusts were forming upon a few of them. Around several of the varicella lesions in the skin over the lower part of the abdomen and over the left shoulder posteriorly, there were broad areas of exfoliated skin similar to the one seen on admission. Some of these areas were confluent, giving the appearance of a superficial burn. Boric ointment was applied to these areas. There was a purulent discharge from the left ear, tenderness over the left mastoid process, and a red, bulging right ear drum. The left ear was irrigated with 2 per cent boric solution.

Feb. 9. The temperature had risen during the night to 102.6°F. A few new varicella lesions had appeared, particularly on the forearms and legs. Many of the older ones were drying. Over the trunk, the edges of the drying lesions appeared red and infected. Over the lower abdomen there was a considerable increase in the size of the areas of exfoliated skin. Infected varicella lesions appeared to be the centers of these areas. Ammoniated mercury ointment (3 per

cent) was applied to all infected varicella lesions and to the areas of exfoliated skin. The temperature rose to 103.6°F. at 4 p.m. The discharge from the left ear was not profuse, but the right ear drum was red and bulging. Both drums were punctured. The resultant discharge from both ears, however, was slight, and the otitis did not appear to be responsible for the fever.

Feb. 10. The temperature was 104°F. at midnight. W. B. C. 27,600. The child appeared worse. There was an area of exfoliated skin on the left cheek. Several new areas of skin on both shoulders posteriorly had become involved. Over the lower abdomen, both groins, and the anterior surface of the thighs, the lesions were so extensive that little normal skin remained. On the anterior surface of the right thigh a bleb was observed to increase rapidly in size until it was 2 to 3 cm. in diameter. It appeared to contain yellowish fluid, was thin-walled, and sagged as the fluid increased. There were similar blebs on the anterior surface of the left thigh and on the inner surface of the left leg below the knee. Many of the chicken-pox lesions were not pustular. Fig. 1 shows the extent and character of the different types of lesions. Under aseptic conditions, yellow, turbid fluid was removed from the blebs through a capillary pipette. A stained preparation of the fluid revealed numerous pus cells and Gram-positive cocci, many of which had been phagocyted. Cultures on blood-agar plates from the pus showed a hemolytic *Staphylococcus aureus*.

The number of blebs was increasing rapidly. The ammoniated mercury apparently was unable to prevent extension of the infection. We then tried gentian violet. The child was thoroughly cleansed with soap and water, thus breaking the blebs and removing all crusts. All areas of exfoliated skin and other lesions suspected of being secondarily infected were painted with a 5-per-cent solution of gentian violet in 20-per-cent alcohol. This was allowed to dry. The body was then covered with olive oil, and the child laid naked on a cotton pad in a room kept at a temperature of 85°F.

Feb. 11. At 8 a.m. the temperature was 104.2°F., pulse 154, respirations 40. W. B. C. 26,800. The child was irritable, constantly rolled its head from side to side, but took liquids well. There were no signs of meningitis. The lungs were clear. The abdomen was not distended. The liver and spleen were not palpable. There was no evidence of abscesses. There were two small areas of freshly exfoliated skin on the left buttock and a few small blebs had appeared along the edge of one of the painted areas on the abdomen. No other evidence of the spread of the staphylococcus infection was observable. Gentian violet was again applied as before to both old and fresh lesions. Fig. 2 shows the appearance of the patient after being painted with gentian violet.

Feb. 12. At 8 a.m. the temperature was 104.6°F., pulse 150, respirations 56. The child's condition appeared much the same as on the previous day. There was no cough. The rapid respiration suggested a pneumonia, but no involvement of the lungs was detected by physical examination. There had been no extension of the infection of the skin since the previous day. The gentian violet

FIG. 1. PHOTOGRAPH OF THE PATIENT SHOWING APPEARANCE OF THE LESIONS ON FEBRUARY 10TH BEFORE THE APPLICATION OF GENTIAN VIOLET

The arrows indicate intact blebs

FIG. 2. PHOTOGRAPH OF THE PATIENT ON FEBRUARY 11TH, AFTER THE APPLICATION OF GENTIAN VIOLET

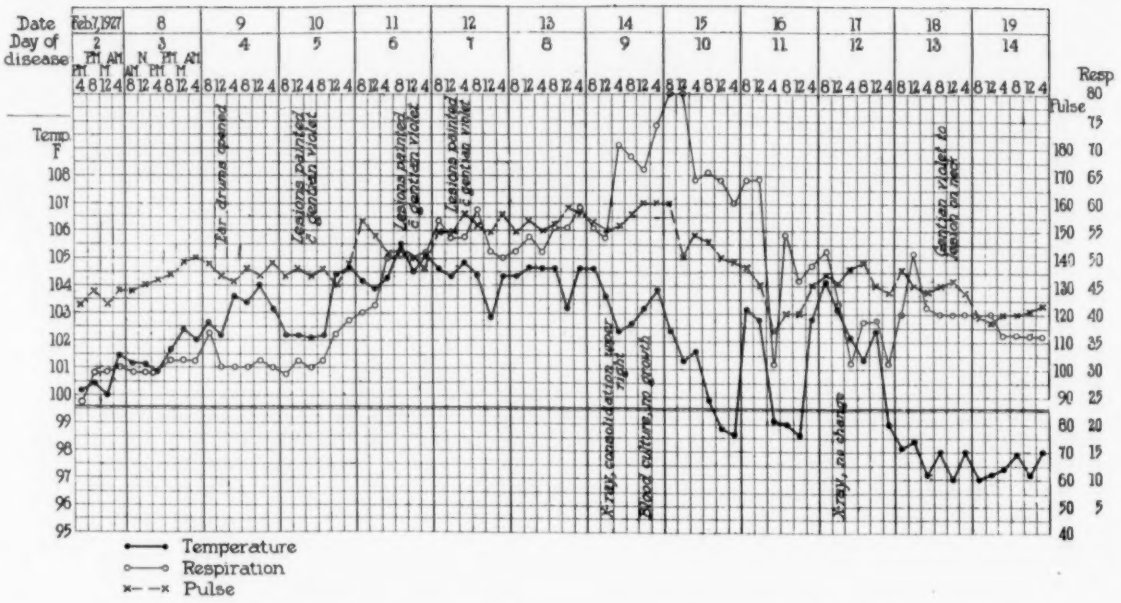

CHART 1. CHART SHOWING COURSE OF THE PATIENT'S TEMPERATURE, PULSE, AND RESPIRATIONS

For convenience the latter part of chart is abbreviated

had been rubbed from some of the lesions on the back. It was applied to these areas only.

Feb. 13. His general condition appeared unchanged. The child had developed a cough on Feb. 12, which had persisted. There were signs of consolidation over the upper lobe of the right lung posteriorly. A diagnosis of lobar pneumonia was made at this time. There had been no extension of the infection of the skin. Gentian violet was applied again to moist lesions from which it had been partially removed.

Feb. 14. The temperature, pulse, and respiration had remained the same. The child appeared no worse. The diagnosis of lobar pneumonia was confirmed by X-ray. A few moist lesions were painted again with gentian violet. The remaining lesions were drying. A blood culture made on this day remained sterile. From the sputum a Group IV pneumococcus was isolated.

Feb. 17. The X-ray showed that there had been no extension of the consolidation of the lungs.

Feb. 18. The temperature had fallen to normal, but the pulse and respirations remained rapid. The course of the temperature, pulse, and respiration from February 14 to 18 is recorded on Chart 1. The skin lesions had continued to heal. The few moist areas had been painted each day with gentian violet. The majority of the lesions now exhibited dry surfaces with normal epidermis encroaching upon their edges. Below and to the left of the occiput an area of freshly exfoliated skin, 2.5 cm. in diameter, was noted. This lesion appeared to be similar to the ones which were widespread a week previously. It was painted with gentian violet.

March 8. The temperature had remained subnormal for the previous three weeks. The pulse and respirations had slowly returned to normal. Signs of consolidation had disappeared, and there had been no fresh skin lesions. The lesion noticed on February 18th had healed, after being painted twice with gentian violet. The distribution of the areas of exfoliated skin was still evidenced by the lighter color of their healed surface as contrasted with that of the uninvolved skin and by the small central depressions, the sites of the original chicken-pox lesions around which the exfoliation had occurred.

DISCUSSION

From clinical observations alone, Fox (3), in 1864, was able to differentiate impetigo contagiosa from numerous other diseases with which it had been confused. Sabouraud (4) in 1900, maintained that the type of impetigo described by Fox was caused by streptococci, and that the staphylococci that were often found associated with these organisms were secondary invaders. In the same year Gilchrist's (5) investigations led him to conclude that *Streptococcus pyogenes* is

the probable cause of the disease. Farley and Knowles (6), in 1921, after investigating 30 cases of impetigo, also concluded that the disease is caused by streptococci and that associated organisms, such as staphylococci and diphtheroids, are secondary invaders. Bommer (7), in 1923, found streptococci and staphylococci, either alone or together, in impetiginous lesions. Smith and Burky (8), in 1924, in 9 cases of impetigo, found streptococci alone in 6 and staphylococci alone in 3. They suggest that an unknown specific virus may be the cause of the impetigo and that bacteria may be secondary invaders. In 1926, Balmain (9), in a series of 56 cases of impetigo, found streptococci 43 times (13 times in pure culture), staphylococci 52 times (21 times in pure culture), and diphtheroids 3 times (once in pure culture). From the reports cited above it seems evident that streptococci and staphylococci, either alone or together, are the organisms usually found in impetiginous lesions.

Pemphigus neonatorum has not always been considered to be a type of impetigo contagiosa. Knowles and Munson (10) in 1923, divided cases of impetigo contagiosa into 4 groups, one of which includes the bullous type. They also stated that in the past the bullous type of impetigo contagiosa had usually been classified as pemphigus neonatorum. Most authors now hold that these two conditions are identical. Holt and Howland (11), in discussing pemphigus neonatorum, remark: "A better name is bullous impetigo, for its identity with impetigo contagiosa seen in older patients is now generally admitted." In an epidemic of pemphigus neonatorum described by Schwartz (12) in 1908, staphylococci were invariably found in the bullae. In a similar epidemic in 1914, Cole and Ruh (13) isolated *Staphylococcus aureus* from all cases in which unruptured vesicles were examined. They believe, however, that a differentiation should be made between pemphigus neonatorum and impetigo contagiosa seu vulgaris seu bullosa (streptogenes). This opinion was based upon the differences in the types of cocci which are found in these conditions. Falls (14), in 1917, ascribed the cause of pemphigus neonatorum to *Staphylococcus aureus*. In 1923, Knowles and Munson (10), obtained only *Staphylococcus aureus* from cases of bullous impetigo when the material was taken from intact bullae. These authors concluded that the different types of impetigo are caused by

different strains or varieties of cocci. From these reports one is justified in stating that the majority of observers now believe that pemphigus neonatorum and bullous impetigo are identical and that *Staphylococcus aureus* is the organism most frequently found in the fluid of unruptured bullae.

The standard treatment of impetigo contagiosa consists in the removal of the crusts and the application of a mild antiseptic ointment, ammoniated mercury (3 per cent) being the one generally used. Balmain (9), recommends acriflavine, 1:1,000, in a paraffin emulsion, both for removing the crusts and for sterilizing the lesions. He states that Gray had used the same drug as a lotion (1:1,000) in bullous impetigo. After Churchman (15) had shown that gentian violet has a selective action on Gram-positive cocci, Smith and Burky (8) employed a 5-per-cent solution of the dye in 20-per-cent alcohol in the treatment of impetigo contagiosa. They applied it to the exposed bases of the lesions, covered the painted areas with ointment, and then bandaged them. The bandages were not disturbed for two days. The lesions usually healed within six days. The effectiveness of an ointment of ammoniated mercury (3 per cent) was compared with that of gentian violet. The results showed that in cases caused by streptococci the gentian violet was as effective as the ammoniated mercury, but that gentian violet was more effective in the cases caused by staphylococci.

The picture originally presented by our case was that of a typical attack of chicken-pox with the exception of the discharging ear and the area of exfoliated skin over the lower part of the abdomen. Otitis media is not an uncommon complication of chicken-pox, and there is reason to suppose that the drum in this case had opened spontaneously within a short time of the onset of the chicken-pox, and that the organisms in the purulent discharge had been spread over the whole surface of the body. In this manner fresh foci of infection were established in the sites most favorable for the growth of the organisms, that is, in the medium provided by the chicken-pox lesions. The fact that *Staphylococcus aureus* was recovered from the discharge in the ear and from the fluid of the unruptured blebs is strongly suggestive of the relation of the secondary skin infection to the spreading of the organism from the ear.

Apparently, the secondary skin lesions arose only from sites already occupied by chicken-pox lesions and not from hitherto uninvolved portions of the body surface. The bullae appeared chiefly in the skin over the lower part of the abdomen, groins, inner surfaces of the thighs, and the backs of the shoulders. These regions are subject to pressure and irritation from clothes and posture, and are less exposed to the air than other portions of the body. The formation of the late lesion on the back of the neck was doubtless favored by the child's habit of lying on his back and constantly rolling his head. No bullae appeared on the hands or feet and only two on the face. The picture presented by the large blebs was typical of bullous impetigo complicating varicella.

The number of bullae was rapidly increasing under treatment with 3-per-cent ammoniated mercury. It was feared that the areas of exfoliated skin would soon become so large that, as in extensive burns, the absorption of toxic material might prove fatal. A large portion of the anterior abdominal wall had already been denuded of skin and the steady rise of temperature was thought to be due to the absorption of toxic substances. One application of gentian violet was sufficient to show that the spread of the infection in the skin could be controlled in this manner. After a second complete application of the dye, only occasional reapplications to isolated lesions were necessary. The exfoliation of the skin was very superficial, and the dye could readily come in contact with the organisms. As a result, the lesions, when healed, left only a discoloration and no scarring.

The lobar pneumonia apparently had little effect on the infection in the skin. In fact, as the fever was rising as a result of the pulmonary lesion, and while it remained high, the skin was responding well to treatment. Lobar pneumonia complicating chicken-pox is very rare. It is a primary disease in a large proportion of the cases, and the prognosis is usually good. The child appeared less acutely ill during the height of his pneumonia than when the secondary skin infection was at its acme.

SUMMARY

Our case was one of chicken-pox complicated by bullous impetigo. The bullous lesions contained an organism, *Staphylococcus aureus*,

similar to that obtained from a discharging ear. The impetigo was successfully treated with gentian violet.

REFERENCES

(1) SCHAMBERG, J. F.: Diseases of the Skin and the Eruptive Fevers, Philadelphia, 4th Edition, 1921, 535.
(2) WELCH, W. M., AND SCHAMBERG, J. F.: Acute Contagious Diseases, Philadelphia, 1905, 330.
(3) FOX, W. T.: *Brit. Med. J.*, 1864, i, 467, 495, 553, 607.
(4) SABOURAUD, R.: *Ann. de dermat. et de syph.*, 1900, Series 4, I, 62, 320, 427.
(5) GILCHRIST, T. C.: Contribution to the Science of Medicine by Pupils of W. H. Welch, Baltimore, 1900, 409.
(6) FARLEY, D. L., AND KNOWLES, F. C.: *Arch. Derm. and Syph.*, 1921, iii, 753.
(7) BOMMER, S.: *Derm. Ztschr.*, Berlin, 1923, xxxix, 143.
(8) SMITH, D. T., AND BURKY, E. L.: *Johns Hopkins Hosp. Bull.*, 1924, xxxv, 78.
(9) BALMAIN, A. R.: *Lancet*, 1926, ii, 484.
(10) KNOWLES, F. C., AND MUNSON, H. G.: *Arch. Derm. and Syph.*, 1923, vii, 376.
(11) HOLT, L. E., AND HOWLAND, J.: Diseases of Infancy and Childhood, New York, 8th Edition, 1923, 91.
(12) SCHWARTZ, H. J.: *Bull. Lying-In Hosp.*, New York, 1908, v, 1.
(13) COLE, H. N., AND RUH, H. O.: *J. Am. Med. Assn.*, 1914, lxiii, 1159.
(14) FALLS, F. H.: *J. Inf. Dis.*, 1917, xx, 86.
(15) CHURCHMAN, J. W.: *J. Am. Med. Assn.*, 1920, lxxv, 583.

TRANSVERSE LINES IN X-RAY PLATES OF THE LONG BONES OF CHILDREN[1]

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In the course of a study of rickets which has been in progress in New Haven for the past four years we have had occasion to examine large numbers of x-ray plates of the forearms of children, aged from two weeks to five years, and have been struck by the great frequency of occurrence of transverse lines at the lower ends of the radius and ulna. Becoming interested in the possible relationship of these lines to rickets and to other conditions which interfere with growth or nutrition, we performed experiments on animals in the hope of producing transverse lines in their bones and in that way finding out how such lines were formed and what they meant. We have succeeded in producing transverse lines in the bones of animals. The more, however, that we have worked on the problem, the more clearly we have realized that we cannot understand it unless we know how bone is formed and destroyed and what causes it to be destroyed, to grow or to fail to grow. In other words, we cannot solve the problem until we have become conversant with those regulatory mechanisms which make bone adapt its structure so perfectly to the supply of materials available for its nutrition, on the one hand, and the mechanical demands imposed upon it by the rest of the body, on the other.

This paper is intended, primarily, to show the anatomical conditions in the bone which are responsible for the appearance of transverse linear shadows in the image on the x-ray plate. It records, also, observations concerning the occurrence of transverse lines in the x-ray plates of bones of children,—observations which have been made chiefly from the study of the radius and ulna,—and gives a brief statement of our conception of the principles involved in the formation of the lines and of the relationship of rickets to them.

[1] Received for publication August 25, 1927.

OBSERVATIONS ON THE LIVING SUBJECT

If one scans x-ray plates of bones of infants and children for transverse lines, one finds them, or traces of them, so often as to be forced to think that the phenomenon is quite common. In the x-ray pictures of the arms and legs of many children we have discovered several lines at the rapidly growing ends of each long bone, and, in some instances, we have counted as many as twelv.. On the whole, transverse lines are more commonly encountered in older children than in infants, but are seen commonly enough in the latter. We have found them even in a new born baby in whose case, of course, the development must have been intrauterine. They are of much more frequent occurrence in children who have been acutely or chronically sick than in those who have always been well, and are most numerous in those who have had wasting diseases or have been malnourished for long periods of time. The lines are very frequently found in bones which are rarefied, possibly, because more easily visible in them. Lines are particularly common and clearly outlined in children who have suffered from anaemia.

The transverse lines are almost always symmetrically disposed in the skeleton; that is, if they occur in one bone, they will usually be found in an exactly similar situation in the corresponding bone of the other side. A given line may not be quite so clearly outlined in the one as in the other, and, if faint in one bone, may not appear at all in its fellow or be represented only by traces.

The lines are found most commonly and are most conspicuous in those parts of the long bones where growth is taking place with the greatest rapidity, and, conversely, are usually not seen where growth is occurring slowly. For example, they are most numerous and conspicuous at the lower end of the femur, the upper and lower ends of the tibia and fibula, the lower ends of the radius and ulna and the upper end of the humerus; they are seen much less frequently at the upper end of the radius and the lower end of the humerus and perhaps least often at the upper end of the ulna. Since our experience is concerned chiefly with the radius and ulna, we cannot discuss the relative frequency of transverse line formation in the different bones of the extremities; we can merely express the opinion that, ordinarily, the

condition is most finely demonstrated at the lower end of the femur, the upper end of the tibia and the lower end of the radius; it occurs least commonly, if at all, at the upper end of the ulna. Transverse lines are found in the x-ray pictures of ribs after removal from the body, but do not show in the living subject. Undoubtedly, lines are present in the ribs, if they are present in any other bone of the body, and were it not for the technical difficulties involved in photographing them in the living subject, the ribs would be the bones of election for the study of transverse lines because of their flat shape and thinness.

Lines, corresponding to the transverse lines in the long bones, we have seen in the pelvis, in the bodies of the vertebrae and in the nuclei of the epiphyses. In these flat or round bones, the lines lie just inside the outer margins of the centers and are curved exactly as the outlines of ossification of the centers themselves are curved. One sees beautiful curved lines in the nuclei of ossification of the epiphyses of the long limb-bones in some children who have had scurvy, an observation first made by Reyher (1) and communicated to us by Wimberger (2). The line parallels the outline of the nucleus on all sides except the one which faces the shaft; with this latter it coincides. In reality the curved line marks the outline of the nucleus of ossification as it was at an earlier stage of growth, when the scurvy first developed. The line in the nucleus of ossification in cases of scurvy serves to demonstrate how the nucleus grows,—that it expands in all directions save on the side toward the shaft, where it does not grow at all. In the ileum the line parallels the S-shaped curve of the crest.

The transverse lines in the long bones are always, in their turn, exactly parallel to the ends of the shafts. In all bones in which the cartilage-shaft junction curves, the transverse line in the shaft curves in just the same way. At the lower end of the femur, for example, the transverse lines describe the exact undulations of the lower end of the shaft. The parallelism between the lines and the margins at which growth is taking place is an observation of great importance, since it points to the seat of origin of the lines in the growth surfaces.

Though the ends of the shafts of the long bones often appear in x-ray plates as lines, they are of course surfaces, and their character as surfaces is usually revealed in the x-ray plates by an oval, a figure 8, or a loop-shaped outline. The margin nearest to the plate casts

the brightest shadow. The transverse lines in the shafts under discussion show, also, in some cases a double outline corresponding to that of the end of the shaft. The significance of this fact will become clear presently.

At the slowly growing ends of the long bones, the transverse lines, if present at all, always lie close to the cartilage; at the rapidly grow-

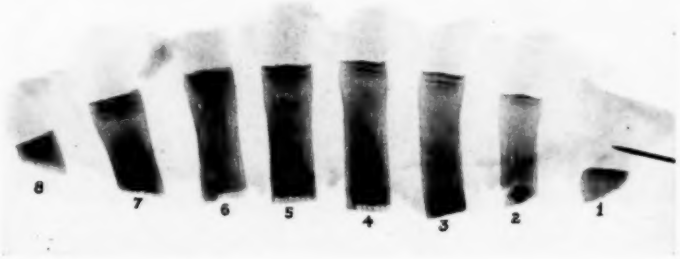

FIG. 1. AN X-RAY PHOTOGRAPH OF A SERIES OF RIBS REMOVED AFTER DEATH FROM A CHILD ABOUT EIGHT MONTHS OLD

Each rib shows a bright line. The lines are parallel to the ends of the ribs. The distance between the bright line and the end of the rib is the shortest in the case of the first rib and increases progressively to the seventh and eighth. The varying distance of the end of the rib from the line shows that growth must have been slowest in the first rib and fastest in the seventh and eighth. The line marking the termination of the fifth rib is doubled near the center. This doubling is due to the fact that the margin of the surface of the end of the rib on the side away from the plate shows, that is, casts a shadow on the plate. If the transverse line in this rib is carefully examined, it, too, will be seen to be doubled and to correspond exactly with the outline of the surface of the end of the rib. This apparent doubling of the transverse line in the x-ray photograph shows clearly that it is produced, not by a line in the substance of the bone, but by a plate-like formation extending through the bone exactly parallel to the terminal surface.

ing ends, on the other hand, they occupy any part of the distal third of the bone, but, as a rule, are better defined near the cartilage. They may lie so close to the end of the shaft that the line formed by the end appears doubled. Then again, they may lie in such proximity to each other in the shaft substance as to be separated by no more than their

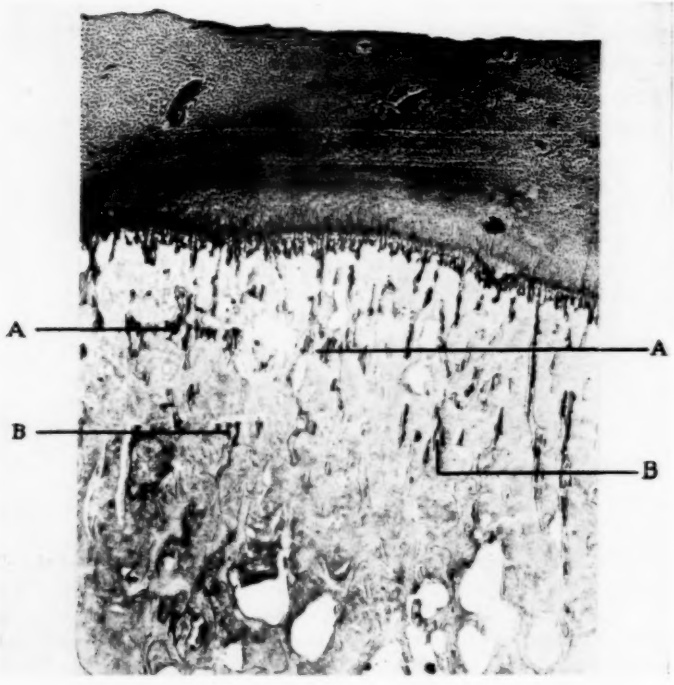

FIG. 2. A PHOTOMICROGRAPH OF A SECTION PREPARED FOR MICROSCOPIC STUDY FROM ONE OF THE RIBS OF THE SERIES SHOWN IN FIGURE 1

Above is the cartilage. At the points marked *A* can be seen fragments of the lattice formation in the shaft which gave rise to the bright line seen in the x-ray photograph. The fragments consist of two groups of trabeculae which in their process of formation at the cartilage-shaft border became joined in the horizontal plane. At the points marked *B* are indications of another faint line just visible in the x-ray photograph beneath the bright line. Sections prepared for histological study, on account of their thinness, often fail to show the lattice formation in the bone substance which is responsible for the transverse line in the plate, even though with the binocular microscope and a cleared specimen it can be seen to be well developed.

own width; or, they may be widely separated. Near the marrow cavity the lines tend to become fainter and to be imperfectly developed. In some instances the lines are complete, extending across the bone from cortex to cortex; in other instances they are deficient at one or at several points. In the lower end of the radius it is not uncommon to find them complete in the inner, or ulnar, two-thirds, but wanting or imperfectly represented in the outer third. We have seen all kinds of interruptions in continuity, from lines defective at one or both ends to lines so full of gaps as to consist only of a series of points arranged in a line across the bone. The lines may also display great differences in density. Some are exceedingly bright; some are just visible. Their thickness varies from that of a hair to the mark of a soft pencil.

FIG. 3 X-RAY PHOTOGRAPH OF THE LOWER ENDS OF THE RADIUS AND ULNA OF A BABY DYING A FEW WEEKS AFTER BIRTH

In both bones transverse lines can be seen close to the ends of the shaft

As one follows the disappearance of the transverse lines in a consecutive series of x-ray plates, one sees that, although the line may become fainter as a whole, it does not fade equally at all points. Gaps form which become wider and more numerous, converting the line to a few fragments or points and finally, perhaps, reducing it to a single point in the finer structure of the shaft.

We have been able in some instances through the examination of plates in series to identify transverse lines in the process of formation at the cartilage-shaft border.

Transverse lines never move in relation to each other in the course of growth and therefore afford a beautiful natural proof that interstitial growth of bone does not exist.

When one examines with a lens a complete transverse line in an

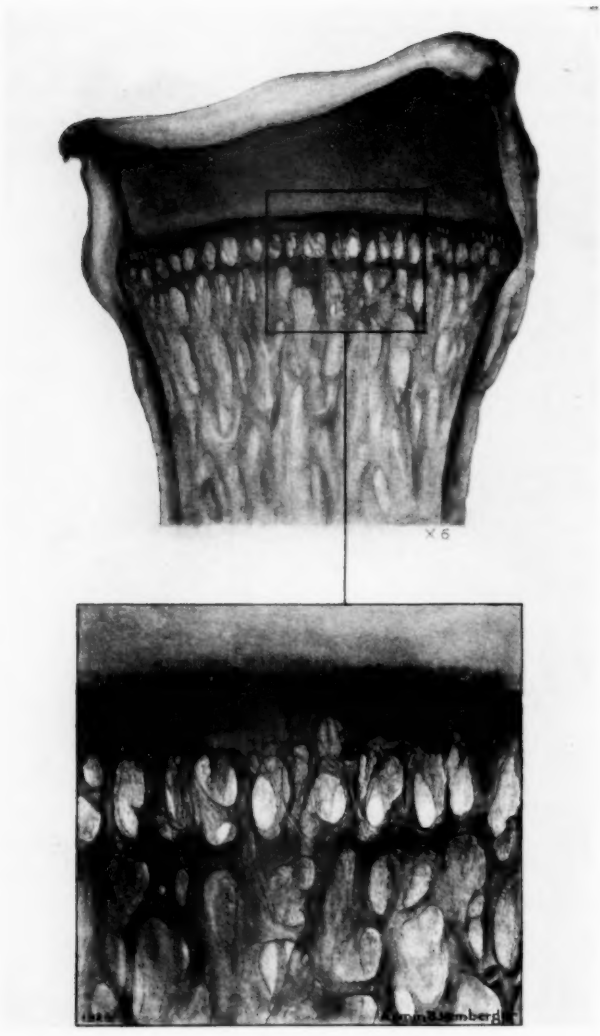

FIG. 4. A DRAWING FROM THE RADIUS SHOWN IN FIGURE 3

The transverse lattice formation which is the cause of the transverse line in the x-ray plate is beautifully demonstrated. The drawing was made under the binocular dissecting microscope from a preparation cleared in oil of wintergreen according to the method of Spalteholz.

x-ray plate, one sees that it is always slightly irregular. In some instances the units of its composition are apparent. They are minute points and longitudinally directed lines which together form a row

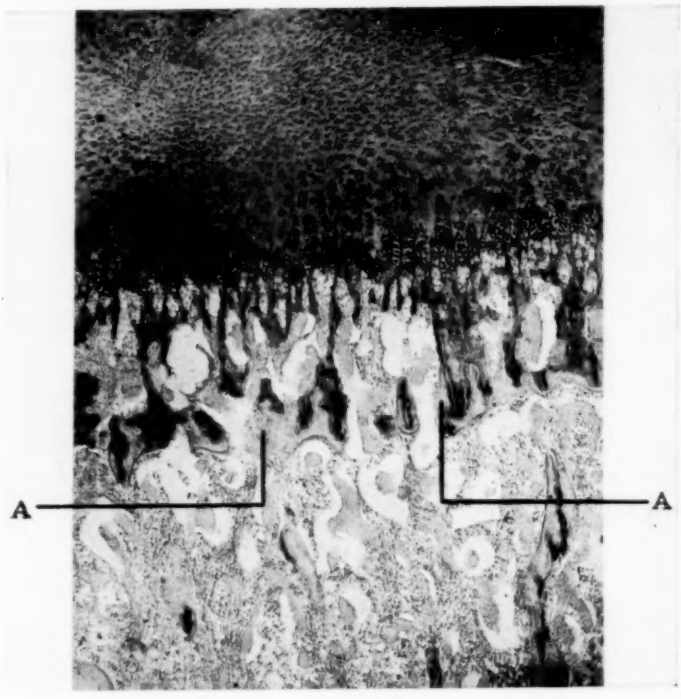

FIG. 5. A MICROPHOTOGRAPH OF A SECTION PREPARED FOR MICROSCOPIC STUDY FROM THE RADIUS SHOWN IN FIGURE 3

The transverse union of the trabeculae which is at the basis of the formation of the transverse sheet of lattice is well shown, but not so well as in the drawing in Figure 4.

across the bone and can be seen to be continuous here and there with the trabeculae above and below.

Transverse lines are frequently found to alternate with transverse linear zones of apparent rarefaction. It is often difficult to make out

whether these lighter linear zones are caused by true rarefaction of the bone substance or whether they are the result of contrast with the dense lines above and below. These transverse lines of rarefaction like the transverse lines of increased density are exactly parallel to the end of the shaft.

In bones showing transverse lines one often finds transverse zones several millimeters in depth where the cancellous tissue has an abnormally coarse or abnormally fine structure. These zones are often interspersed between the lines.

TECHNIQUE

The bones studied were hardened in formalin and cut with a sharp knife longitudinally in a plane parallel to that shown in the x-ray plate, in order that the finer structure might be compared directly with the image studied. The cut surface was first looked at with the naked eye and then placed under water and examined with the binocular dissecting microscope. In all cases slices from the cut surface were taken, decalcified by the formalin-formic-acid method recommended by Schmorl (3), sectioned in celloidin and stained with haemotoxylin and eosin. In some instances the bones were cleared according to the method of Spalteholz (4) and studied in oil of wintergreen under the dissecting microscope. This last procedure gives the most satisfactory demonstration of the alteration in the structure of the bone which is responsible for the transverse line in the x-ray photograph.

THE ANATOMICAL CONDITION IN THE BONE RESPONSIBLE FOR THE TRANSVERSE LINE IN THE X-RAY PLATE

When the cut surface of a bone which casts the characteristic linear shadow in the x-ray plate is inspected, the formation responsible for the shadow is visible to the naked eye but cannot be seen well without magnification. If one examines under the binocular microscope a preparation rendered transparent, one sees that the formation which appears as the transverse line in the x-ray plate does not rest in the bone substance in the form of a line but in the form of a stratum, about 1 mm. in thickness, which lies in a plane at right angles to the axis of the shaft and exactly parallel to the surface of the end of the shaft, or to the zone of provisional calcification of the cartilage, which is saying the same thing. One sees further that at the level in question many of the trabeculae are enlarged or send to neighboring trabeculae cross branches or

bridges. One perceives, at once, that the formation in the bone responsible for the line in the x-ray plate consists in an irregular latticework which extends transversely across the bone, and that the lattice is produced by the enlargement and cross branching of the trabeculae. Since the lattice is so exactly parallel to the surface of the end of the shaft, one knows that it must mark a former growth level of the bone.

Above and below this lattice formation the trabeculae have the usual shape and lie in the longitudinal axis of the bone, parallel to each other. Some of these in their passage through the lattice have undergone enlargement or branching. The zones immediately above and below the lattice may have the usual number of trabeculae or they may have very few. In other words, the adjacent bone may

FIG 6. THE X-RAY PHOTOGRAPH OF THE LOWER ENDS OF THE RADIUS AND ULNA OF A THREE-WEEKS-OLD BABY

Just underneath the end of the bone is a bright line.

show true rarefaction. Through the apertures of the lattice pass the blood vessels and cells of the marrow cavity. The latter, however, are demonstrable only in the stained histological preparations.

Sections of the bone suitable for histological study, 20 to 40 microns thick, often fail to reveal the true nature of the lattice formation on account of their thinness. In general, microscopic study of stained sections shows at the level of the transverse line trabeculae ending in variously shaped enlargements comparable in their irregularity of outline to the heads of shillelahs. Some send forks or branches laterally, which end abruptly, or actually join adjacent trabeculae. If the lattice formation is sufficiently dense, the trabeculae, even in microscopic sections, exhibit beautiful transverse bridging which extends com-

pletely or almost completely across the bone. If this formation is poorly developed, histological preparations may fail to indicate the actual condition.

The transverse line in the x-ray plate is thus seen to be caused by

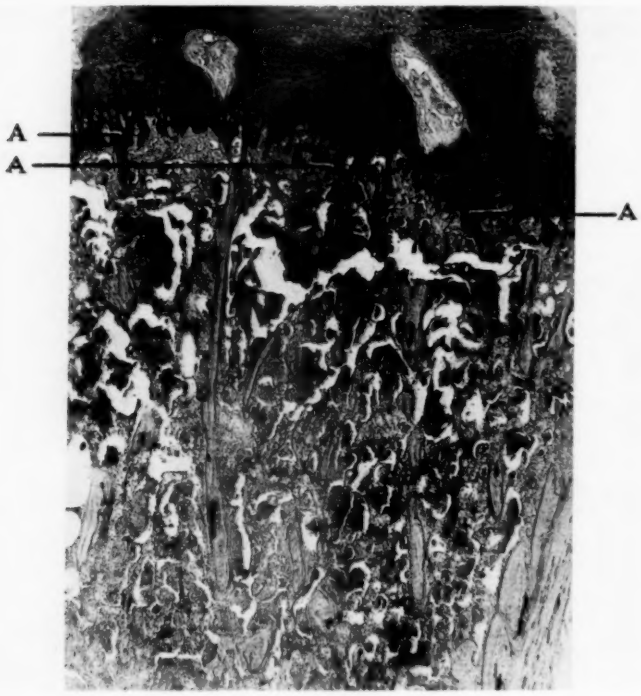

FIG. 7. MICROFHOTOGRAPH OF A HISTOLOGICAL PREPARATION FROM THE RADIUS SHOWN IN FIGURE 6

At three points marked *A* fragments of transverse lattice can be seen. They lie just beneath the cartilage-shaft junction where they were formed. If the child had lived and growth had been renewed, these fragments would have been left behind in the shaft as parts of a transversely arranged sheet of lattice. The fragments are composed almost entirely of calcified ground substance of the cartilage, as is indicated by their blackness in the microphotograph. The calcified ground substance stains a deep blue with haemotoxylin; the lamellated bone remains gray.

the superimposition of the shadows of the enlargements and cross branches of the trabeculae. These latter lie in the substance of the bone in a plane perpendicular to the main axis and parallel, or nearly so, to the X-rays, and therefore obstruct the rays by virtue of their total lateral mass. The reason for the peculiar interrupted or punctate character of the linear shadow in the x-ray plate is apparent, now that its anatomical substratum is understood.

When several transverse lines appear in the x-ray plate, one may think of the bone as containing a corresponding number of horizontal fenestrated plates, parallel to one another and to the provisional zone of calcification of the cartilage. These plates may be no more than their own thickness apart or they may be widely separated; they may be complete, or incomplete, poorly developed or well developed, etc., as necessarily follows from the description of the lines in the x-ray photographs.

THE GROWTH IN LENGTH OF LONG BONES

Growth in length of the long bones takes place as the result of growth of the cartilage. The cells in the generating portion of the proliferative cartilage multiply continually during the periods of longitudinal growth of the bone, and keep giving off cells toward the shaft and away from the nucleus of ossification. Growth at the upper end of the tibia, for example, is due to the fact that the generative portion of the proliferative cartilage keeps lifting itself by continually producing cells from its under surface which accumulate between its under surface and the upper end of the shaft until converted into bone. The marrow elements are powerless to cause the bone to increase in length, as is so beautifully demonstrated in chondrodystrophy. What the blood vessels and other building forces of the marrow do is to turn the material furnished by the cartilage into bone. As fast as the cartilage supplies its cells to the waiting blood vessels and cells of the shaft, so fast do these latter convert the cartilaginous material into shaft. Naturally, those cartilage cells nearest the shaft are the oldest. These oldest cells have probably undergone some chemical or physical change in the process of waiting, which gives to their exoplasm, the homogeneous ground substance which fills in the spaces between the cell columns, some special power to attract lime salts from the blood.

At any rate, in the ground substance surrounding these oldest cartilage cells which lie next to the shaft, lime salts are deposited. The blood vessels with their enveloping cells from the marrow cavity pene-

FIG. 8. X-RAY PICTURES OF THE RADIUS AND ULNA IN A CASE OF MODERATE RICKETS

At the lower end of the radius are three lines which are parallel to each other, but not parallel to the end of the shaft. The lines lie just beneath the rachitic metaphysis. They are separated from each other by scarcely more than their own thickness. The reason that the lines are not parallel to the end of the shaft is because the proliferative cartilage changed its shape after the lines were formed.

trate the spaces which intervene between the calcified ground substance of the cartilage and either free or destroy the cartilage cells which lie in them. They then begin the process of incasing the calcified ground substance with bone. Since the cartilage cells

close to the bone lie in parallel columns in a bed of ground substance, the general plan followed in the conversion of cartilage into bone can be visualized by comparing the cartilage to a honeycomb. Lime salts are first deposited in those portions of the comb of cartilaginous ground substance which lie in immediate proximity to the blood vessels of the shaft; the blood vessels and their accompanying marrow cells then enter the interior of the cells of the comb which contain the cartilage-cell columns, liberate or destroy the lowermost cartilage cells which have become ready (ripe) and begin the process of layering the comb walls with bone. The deposition of lime salts in the comb of ground substance always takes place just ahead of the advance of the vessels and marrow cells. These latter do not turn all the parts of the comb of ground substance into bone; some they build into bone, the rest they break through, and sooner or later destroy.

The cartilage leads in the process of longitudinal growth, the shaft follows. The cartilage furnishes to the shaft design and, at the same time, material on which to build, in the form of the longitudinally arranged hollow cylinders of calcified intercellular ground substance. In consequence, the trabeculae of the shaft have their characteristic parallel arrangement in the long axis. Parts of the material the shaft builds into bone; other parts, which presumably have already served their purpose when they emerge from the cartilage, it destroys.

THE MODIFICATIONS OF GROWTHS WHICH ARE THE CAUSE OF THE TRANSVERSE LINES IN THE X-RAY PLATES

We are not certain in regard to various details in the development of transversely disposed trabeculae which are the basis of lattice formation in bones, but we believe that the general principles are clear. The formation has its origin at the cartilage-shaft junction. The growth of the cartilage is suddenly slowed or stops altogether. As a result, the bone-building energy of the shaft concentrates at a single level or at very slowly changing levels and discharges itself in a more lateral direction than usual. The result is the formation of trabeculae which extend transversely or are actually joined to neighboring trabeculae by cross branches. When growth of the cartilage becomes

reawakened, trabeculae with the ordinary parallel longitudinal arrangement are again formed and the lattice is left behind in the shaft.

Under ordinary conditions, when the rate of growth is essentially constant, the depth of the proliferative zone of cartilage, we believe, remains constant; that is, the end of the shaft remains at the same distance from the beginning of the generative zone of the proliferative cartilage. When the growth of the cartilage slows or stops altogether, the blood vessels and marrow cells continue to advance through the ripe portions of the cartilage till they can go no further, and convert the latter into shaft. The end of the shaft, in consequence, moves to a position closer to the generating layer of the cartilage, and the depth of the proliferative zone is greatly shortened.

Cessation of growth of the cartilage can easily be produced in the rat by insufficient or defective nourishment. If young rats, about 21 days old, are made to fast for from two to five days and the cartilage-shaft regions are studied, it is found that the generation of new cartilage has become greatly retarded or has stopped altogether on or before the third day, and that the marrow elements have by that time penetrated well into the proliferative zone, destroying or liberating the cartilage cells and exposing the columns of calcified ground substance which lay between them. They then halt, because they have reached cartilage cells which are unready and, therefore, resistant, blocking further progress. As a result, the proliferative cartilage is reduced to a thin strip one-third or less of its original depth, and the lime salts extend into it only a very short distance.

If the animals are kept upon a defective diet which permits them to live but not to grow, or to grow only very slowly, the deposition of lime salts in the thin layer of cartilage bordering on the shaft becomes very dense. The lime salts not only fill the ground substance of the cartilage between the columns of cells but extend in laterally between the individual cells of the columns. We have often seen in the thin zones of provisional calcification in both animals and children whose growth had almost stopped, lime salt deposits so dense that in the haemotoxylin and eosin stained sections the zones appeared as continuous narrow dark blue bands marking the cartilage-shaft border. The cartilage cells lying in the bands were so completely surrounded by the lime salt deposits as to look like eyes.

Along these shallow zones of dense provisional calcification the osteoblasts settle and begin to layer the calcified ground substance

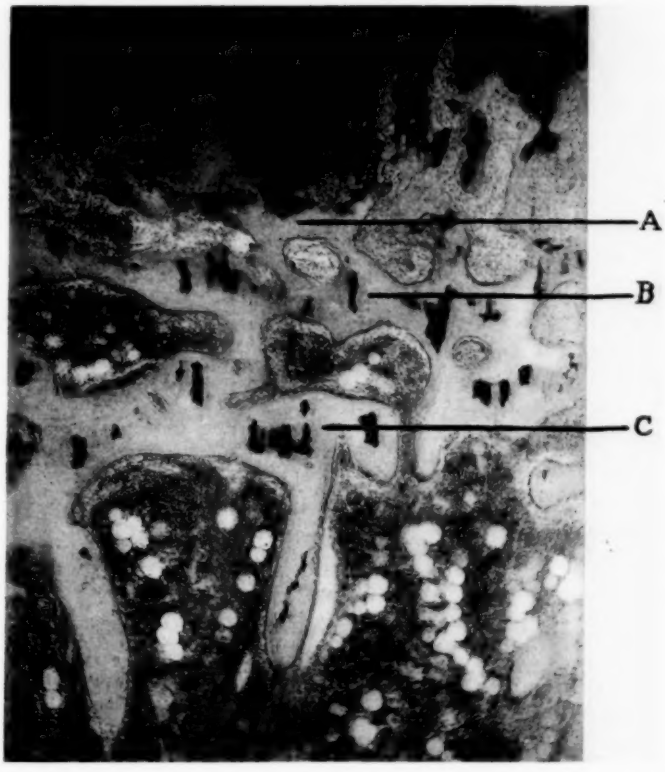

FIG. 9. MICROPHOTOGRAPH OF A HISTOLOGICAL SECTION FROM THE RADIUS SHOWN IN FIGURE 8

The formations in the bones responsible for the lines are well shown at *A*, *B*, and *C*. Evidences of rickets are only too apparent. The cartilage above is extensively invaded and the lime-salt deposits in it are fragmentary.

with bone, entering the gaps and spaces formerly occupied by the cartilage cells, filling them in and making the beginning of transverse trabecular formation.

For some time we thought that the dense deposits of lime salts in the provisional zone of calcification caused the formation of the horizontal stratum of lattice in the shaft. In extreme cases the lime salt deposits formed a dense network at the cartilage-shaft junction. We conceived of these rich deposits at the junctions as perhaps exerting a chemotactic influence on the osteoblasts, causing them to settle there in large numbers. We were forced, however, to question this idea when we encountered characteristic, horizontally running strata of lattice in the bones of children suffering from rickets, in which lime salt deposits were scanty, and were compelled to think rather that it was the ground substance of the cartilage itself, in which the lime salt deposition takes place, that exercised the attraction. We believe it probable, when the cartilage of the proliferative zone remains for some time in contact with the blood vessels and tissues of the shaft, as is necessarily the case when the growth of the cartilage becomes very slow, that its contact layer of cells develops more than the usual amount of exoplasm (ground substance). This superabundant ground substance gives rise to a closely meshed, heavy, cartilaginous network on which the shaft deposits and builds bone. So far as we are aware, lamellar bone, of which the trabeculae of the shaft are entirely composed, develops only about a nucleus of cartilage or on the surface of existing bone. The bony lattice cannot spring up spontaneously; it must be formed on a cartilage basis. The development of a close network or framework across the surface of the end of the shaft, composed of cartilaginous ground substance, is, we believe, the first step in the development of the bony lattice.

Of course, the development of transverse formations in bone must be dependent on combinations of fortuitous conditions, for we cannot suppose that always, when the growth of the cartilage suddenly slows or stops, abnormally dense growth zones are produced in the bone. In histological sections of bones showing all the signs that growth in length had ceased, we have repeatedly found provisional zones of calcification which were densely infiltrated with lime salts but had no bone formations beneath them, or at most only a few short trabeculae. If slow growth of the cartilage were all that is required for the development of horizontally directed trabeculae, we might expect to find the latter regularly at the slowly growing ends of the long bones and as a

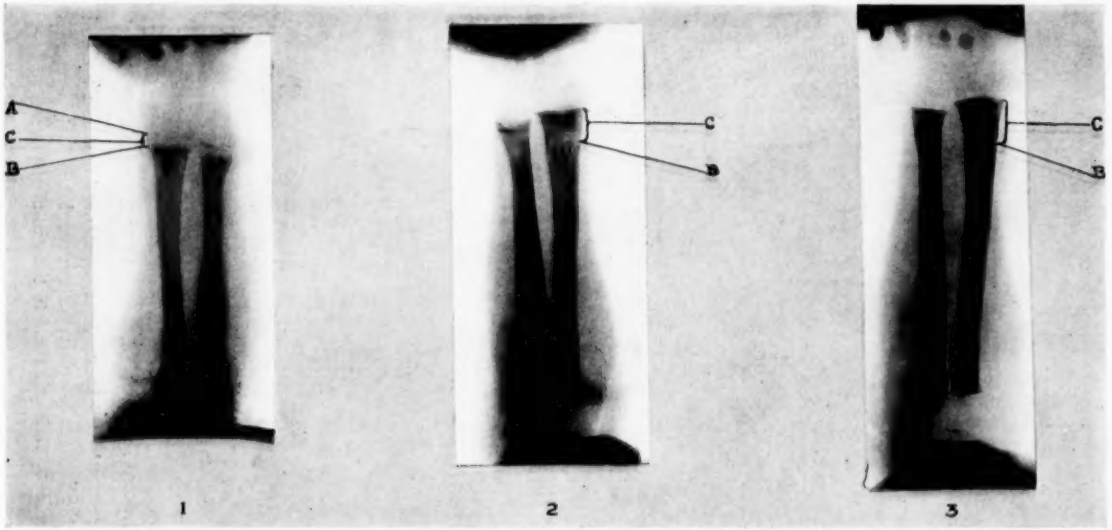

FIG. 10. X-RAY PHOTOGRAPHS OF RADIUS AND ULNA TAKEN IN SERIES AT 14, 17 AND 19 MONTHS, SHOWING HEALING RICKETS

No. 1 shows severe rickets. Line *A* is produced by deposit of lime salts at top of rachitic metaphysis (*C*). This line is the first x-ray evidence of healing of the rachitic process. Line *B* was formed before onset of the rachitic process. No. 2 shows advanced healing of rachitic process with dense zone (*C*) characteristic of healed rickets. Line *B* can still be seen marking the end of the shaft before the rachitic process began. No. 3 shows healed rickets. Zone *C* still shows but is less clearly demarcated. Line *B* still persists, though faint.

FIG. 11. X-RAY PHOTOGRAPHS OF RADIUS AND ULNA OF CHILD OF 26 MONTHS SHOWING SEVERAL TRANSVERSE LINES IN THE RADIUS

Some of these lines are more dense than others, especially those near the end of the radius. Some of the lines are complete, others extend only part way across bone. They are all parallel to each other and to the end of the bone.

regular occurrence at the ends of all long bones as growth in them draws to a close. As a matter of fact, at the slow-growing ends of bones a few laterally disposed trabeculae are very commonly found, and films or layers of new-formed bone extending horizontally along the border of the calcified cartilage for short distances are by no means rare. The slowly growing cartilages of the long bones do, therefore, exhibit some slight tendency to form trabeculae which extend crosswise or at least out of the longitudinal axis. But the frequent occurrence of fully developed transverse lattice formations beneath the rapidly growing epiphyses suggests that an essential condition is the arrest in the forward advance of a powerful growth force and its lateral diversion along the sheet of cartilaginous network which covers the terminal surface of the shaft. As to what this growth force in the shaft may be or on what it may depend, we can only speculate. It is conceivable, however, that it is connected in some way with the circulation.

We are under the impression that transverse lattice formation in the long bones takes place with considerable rapidity, after the growth of the cartilage has been suddenly slowed or arrested. Accordingly, we have wondered if it might not be correct to think of the growth forces in the shaft immediately beneath the cartilage, as being under a momentum or tension which, after the outlet in the longitudinal direction has become suddenly closed by the cessation in the formation of new cartilage, spends itself rapidly along the under surface of the cartilage in the formation of the bony lattice. The bony lattice might then be regarded as the product of the temporary dissociation or lag between the growth forces of the cartilage and those at the end of the shaft, the former halting, the latter for a time continuing. The sharp transitions from transverse lattice to trabeculae with a normal longitudinal arrangement, and the sheet-like thinness of the lattice formations can be most easily explained in this way. If lattice formations were the result of coincident slowing in the growth of cartilage and shaft, which seems to be the only possible alternative, one would expect to find the lattice formations varying greatly in thickness—the thickness being proportional in a general way to the length of the periods during which growth was affected; one would also expect to find them merging gradually with

the normal longitudinal structure of the shaft. We do not, however, find thick zones of dense lattice or lattice formations which pass gradually into normal shaft.

THE RELATIONSHIP OF TRANSVERSE LINES TO RICKETS

When in cases of severe protracted rickets the disease finally recedes and lime salts are again deposited in the bones, the earliest clinical sign is the development in the x-ray plate of a transverse line across the top of the rachitic zone beyond the end of the shaft. When rachitic bones casting this shadow are examined under the microscope, the line of lime-salt deposit is seen to extend across the cartilage at about the level at which the zone of provisional calcification would have lain had rickets never intervened, an interesting observation made years ago by Schmorl (5). This linear shadow, marking the top of the rachitic metaphysis, is produced, not by a line of calcium phosphate, but by a whole sheet of it. Since this sheet of lime-salt deposit rests on edge when the bone is laid on its side against the x-ray plate, the shadow is linear. Though this deposit of lime salts in the restored zone of provisional calcification does cast a transverse line in the x-ray plate, it does not correspond, either as it exists at the time when first visible or in its subsequent fate, to the transverse formations in the substance of the bones which cast linear shadows. It is in reality nothing more than the normal zone of provisional calcification of the cartilage which has been suddenly restored, and owes its visibility in the x-ray plate to the fact that the rachitic zone between it and the end of the shaft, which is almost free from lime salts, casts a feeble shadow and thus allows the provisional zone to show by contrast. This zone of provisional calcification stands out very clearly in the x-ray pictures of completely calcified (non-rachitic) bones, when they are rarefied. In the x-ray pictures of rachitic bones this zone is visible for only a short period of time, because the contrast is soon effaced as the underlying rachitic zone rapidly becomes impregnated with lime salts. From the histological standpoint, also, this zone of provisional calcification which marked the advent of the healing process soon loses its identity, inasmuch as it quickly becomes organized into shaft and new provisional zones keep forming distally to it, as growth continues.

True lattice formations of the kind we have been describing do occur in bones showing advanced rickets, but do not always stand out clearly in the x-ray plate, perhaps because of the scanty amount of lime salts which the trabeculae responsible for them contain. It is not very uncommon in cases of severe rickets to find at the rapidly growing ends of the long bones near the termination of the shaft, one, two, or three transverse lines. When one examines the cut surfaces of rachitic bones showing these lines in the shaft with the binocular microscope, one finds that the formation differs in no respect from that already described. Its origin in the subjects of severe rickets is undoubtedly the same as under conditions in which the mineral metabolism is normal.

It is interesting that in severe rickets these lines are not always parallel to the apparent end of the shaft in the x-ray or to each other, a fact which is explained by the softness of the bone in rickets which permits of uneven compression of intervening tissues, and by the change in contour of the generating zone of cartilage which, as the rachitic process advances, becomes increasingly convex along its shaft surface, while the end of the shaft becomes increasingly concave.

The characteristic situation of these transverse lattice formations in rachitic bones close to the rachitic metaphysis suggests that disturbance in growth often marks the approach of the rachitic state.

In the portion of the rachitic metaphysis near the shaft it is common to find non-calcified trabeculae directed transversely across the bone, and in general throughout the rachitic metaphysis is it characteristic to find an interlacing mass of trabeculae curving about without rule or regulation in all directions. If it were possible in advanced rickets to pull away or to separate the rachitic cartilage from the rest of the rachitic metaphysis, one would see that the surface of the cartilage, thus exposed, presented the most extreme irregularity and that it interdigitated with the rest of the metaphysis by processes of all shapes and lengths. The irregular interdigitating surface of this mass of cartilage keeps degenerating and giving off thin layers of osteoid tissue (bone tissue devoid of lime salts) along various points of contact with the blood vessels and cellular elements which have penetrated from the shaft. These layers of osteoid lie parallel to and have all the bends and configurations of those parts of the surfaces

from which they came. The trabeculae of the rachitic metaphysis which are seen in histological preparations are these osteoid layers in cross section. Since their disorderly arrangement merely reflects the chaotic state of the cartilage whose growth is so completely disordered as the result of the rachitic process, it is no wonder that their arrangement is lawless. Since the metaphysis is soft and is subject to compression, the tendency is for some of these osteoid trabeculae near to the shaft to be flattened out into the horizontal direction.

When rickets completely heals, the former diseased area in the bone never leaves as a residuum in the x-ray plate a line, as is often supposed, but always a transverse band of increased density. This band represents that portion of the bone which was formed during the rachitic process and is always a transverse disc several millimeters to 1 to 2 centimeters in thickness. It is packed with trabeculae that do not have any definite arrangement, and contains large quantities of lime deposits. It casts in consequence a heavy, dense shadow without distinctive pattern in contrast to the orderly pattern left by the longitudinally running network of trabeculae in the normal parts of the bone. This transverse band, the tell-tale of rickets, may occasionally be recognized in the shaft for one to two years after the rickets has ceased.

TRANSVERSE LINES OF RAREFACTION IN THE X-RAY PLATE

Transverse lines of rarefaction are not so numerous or definite as transverse lines of increased density.

One might suppose that, if retardations in the growth of the cartilage cause thin zones of increased density to develop in the shaft, the opposite, namely, thin transversely running zones of rarefaction, might be caused by excessively rapid growth. In the bones, however, which we have examined, the lines of rarefaction have been characterized, not by many thin, longitudinally arranged trabeculae, as might be expected if the growth had been rapid, but by wide gaps in the trabecular formation. The columns of calcified intercellular ground substance either had not been furnished by the cartilage to the shaft, or had been destroyed as they emerged from the retreating cartilage. Presumably the columns of calcified substance were formed by the cartilage and at once removed by the shaft. Transverse zones

of rarefaction are apparently the opposite of zones of increased density, in the sense that the destructive forces of the shaft were ascendant along large parts of the under surface of the cartilage at the time of their formation. If the activity of the osteoblasts, when zones of increased density are formed at the cartilage border, is explained by the presence of an abundant supply of blood at the border, one might think that the destruction of the columns of intercellular substance which was responsible for the development of the zone might be due to a temporary deficiency in the circulation at the border. But this, of course, is conjecture. Our inability to explain why at one time dense lattice formations developed across the shaft, and at another thin zones of rarefaction were formed, shows the paucity of real knowledge concerning the subject.

THE SIGNIFICANCE OF THE VARIATIONS IN THE FINER STRUCTURE OF BONES SHOWN IN THE X-RAY

The x-ray pictures of the bones of children and animals which have always been in uninterrupted health show the cancellous tissue of the shaft to be homogeneous and permit the inference that growth at the cartilage-shaft junction has been of an even kind and gone forward at a constant rate. As already pointed out, x-ray pictures of the bones of children who have been in poor health or poor states of nutrition often show lines of increased density and of rarefaction arranged one above the other. They may also show broad transverse zones in which the trabecular formation is of a fine texture and others in close proximity in which it is coarse. X-ray studies of bones showing such variations in architecture indicate that the growth force must have been, over a long period of time, in a state of oscillation, now halting, now going forward again, now, as the result of a disturbed relation between supply and demand, forming one kind of product, now another. The study of bones is attended with difficulties on account or their hardness. The bones sometimes reveal, however, by virtue of their hardness, experiences in the history of the organism not visibly recorded elsewhere. Of this we shall write fully later.

REFERENCES

(1) REYHER, P.: Über einige seltenere röntgenologische Befunde bei Barlowscher Krankheit. *Verhandl. d. dtsch. Röntg.-Ges.*, 1911, vii, 32.

(2) WIMBERGER, H.: Klinisch-radiologische Diagnostik von Rachitis, Skorbut und Lues im Kindesalter. *Ergebn. der Inn. Med. u. Kinderheilk.*, 1925, xxviii, 269.

(3) SCHMORL, G.: Die Pathologisch-histologischen Untersuchungsmethoden. Leipzig. 1921.

(4) SPALTEHOLZ, W.: Das Durchsichtigmachen als biologische Arbeitsmethode. Handb. der biol. Arbeitsmeth., her. v. Abderhalden. Abt. 9. Teil 1. Lieferung 71. 1922.

(5) SCHMORL, G.: Pathologische Anatomie der Rachitis. *Ergebn. der Inn. Med. u. Kinderheilk.*, 1909, iv, 403.

THE ACID-BASE COMPOSITION OF URINE FROM AN EPILEPTIC CHILD[1,2]

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Various findings have been reported which suggest the presence in epilepsy of an instability of neutrality regulation. Measurements of plasma pH by Geyelin, Bigwood and Wheatley (1), and further data by Bigwood (2), indicate, at least statistically, a drift to an abnormal degree of alkalinity preceding convulsive seizures. If these observations represent an actual pathogenic factor, it is conceivable that the freedom of epileptics from attacks during periods of fasting may be due to an opposing tendency toward an increase of body fluid acidity produced by the accompanying ketosis. Lennox has recently reported reduction of the number of seizures by administration of acid-producing salts and aggravation of their frequency following ingestion of sodium bicarbonate (3). As regards a locus of defect in reaction regulation, Bisgaard and Noervig (4) have found in urine from epileptics deviation from the pH-ammonia nitrogen—total nitrogen relationship described by Hasselbach (5). This they regard as indicating a "rupture" of the mechanism controlling the process of acid excretion in the urine. Bigwood has also reported measurements which suggest an anomalous excretion of ammonia by epileptics (6).

A disturbance of neutrality regulation, if due to a recurring defect in the process of acid excretion, should produce irregularity in the acid-base composition of the urine. The chief regulatory feature of this process being a correct limitation of the use of fixed base in the conveyance of acids into the urine, it would seem probable that an inaccuracy of adjustment of fixed base to total acid excretion, if present in epilepsy, could be demonstrated by measurements of these

[1] The expense of this study was in large part defrayed by a grant from the New York Epilepsy Committee.

[2] Received for publication Aug. 1, 1927.

two factors in the urine. Favorable conditions of study would, however, be required. An essential one would be an accurate constancy of the composition of the food intake to insure closely stationary daily amounts of fixed base and of acids presenting for excretion in the urine. Measurements would best be obtained from 24-hour specimens of urine in order to avoid post-prandial and diurnal variations of composition. Obviously, however, an inaccuracy of urinary secretion due to a brief manifestation of disease might be adjusted for during a 24-hour period. It would, therefore, be a matter of critical importance that the subject selected present severe and frequently recurring activities of disease during at least 24 hours. There is also the requirement that the periods of attacks be separated by intervals of at least several days.

In this paper are presented measurements of acid-base factors in urine obtained from an epileptic girl eleven years of age. She exhibited the disease to a degree and with a periodicity which excellently suited the plan of study. Frequent and severe seizures occurred during periods of about two days' duration and there was complete freedom from attacks through intervals usually lasting nearly a week. This child was placed on a constant diet[3] and a stationary level of fluid intake. After an initial interval of five days, the collection of 24-hour urine specimens was begun and was continued for twenty-nine days. During this period groups of seizures occurred four times, and in each instance occupied approximately two successive days. The individual attacks differed in severity from brief moments of unconsciousness, following a cry, to several minutes of convulsion.

[3] The materials used in the diet and the daily amounts of each were as follows; cereal (oatmeal) 28 gm., bread (salt-poor) 102 gm., eggs 96 gm. (approximately), cane sugar 36 gm., butter (salt-poor) 42 gm., milk 690 cc., and orange juice 112 cc. A palatable saltiness of the cereal was obtained by cooking it in a measured amount of a solution of sodium chloride. Salt-poor bread and butter were used to avoid an irregularity of salt content. The other articles of food, however, provided together an intake of sodium chloride not appreciably less than a usual diet contains.

The diet was completely taken throughout the period of study except on the following days; January 19th, January 27th, February 11th and February 27th. On these days quite half of the food offered was not taken. These refusals in each instance occurred on the second day of a period of seizures.

TABLE I

DAY	VOLUME	pH	ACID EXCRETION (AT pH 7.4)					TITRATABLE ACIDITY	NH_3	FIXED BASE	
			Organic acids	SO_4''	PO_4''	Cl'	Total acid			Indirect	Direct
	cc.		cc. 0.1N	cc. 0.1N	cc. 0.1N	cc. 0.1N	cc. 0.1N	cc. 0.1N	cc. 0.1N	cc. 0.1N	cc. 0.1N
January 16	535	5.6	137	171	300	184	792	152	268	372	359
17	905	5.6	172	224	396	281	1073	191	348	534	529
18	**1280**	**5.2**	**480**	**304**	**364**	**1039**	**2187**	**330**	**354**	**1503**	**1604**
19*†											
20	780	5.8	332	404	531	32	1303	300	654	349	321
21	770	5.8	239	236	344	118	937	171	386	380	389
22	685	5.8	162	198	324	375	1059	167	296	596	594
23	1040	5.7	199	244	360	471	1274	200	307	767	796
24	935	5.7	212	218	324	493	1247	172	296	779	772
25	695	5.5	188	208	301	300	997	180	279	538	561
26	**1500**	**6.0**	**264**	**225**	**363**	**1236**	**2138**	**201**	**339**	**1598**	**1678**
27*	**770**	**6.1**	**204**	**216**	**327**	**450**	**1197**	**152**	**336**	**709**	**748**
28	170	5.7	99	139	211	14	463	110	219	134	150
29	460	5.7	166	184	335	86	771	180	311	280	252
30	740	5.6	199	240	375	354	1168	210	454	504	576
31	500	5.5	139	185	353	214	891	187	362	342	376
February 1	**645**	**5.5**	**176**	**178**	**344**	**418**	**1116**	**193**	**359**	**564**	**543**
2	**1040**	**5.6**	**203**	**221**	**350**	**546**	**1320**	**187**	**399**	**734**	**731**
3	695	5.8	189	235	304	300	1028	156	317	555	552
4	635	5.6	111	173	307	171	762	154	289	319	345
5	535	5.4	127	173	277	289	866	167	286	413	472
6	395	5.5	125	137	237	225	724	127	224	369	339
7	720	5.8	102	115	176	193	586	92	204	290	321
8	835	5.5	144	198	288	289	919	171	335	413	416
9	740	5.6	156	207	336	246	945	180	309	456	472
10	**1140**	**5.6**	**358**	**230**	**303**	**1061**	**1952**	**176**	**338**	**1438**	**1449**
11*	**620**	**5.8**	**156**	**164**	**374**	**375**	**1069**	**186**	**319**	**564**	**591**
12	670	5.8	143	245	256	21	665	133	329	203	206
13	360	5.8	230	350	273	21	874	221	289	355	327
14	825	5.7	172	212	311	300	995	165	307	523	532
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
24	520	5.8	155	256	288	334	1033	144	470	419	419
25	630	5.8	201	218	303	450	1172	156	446	570	558
26	**1030**	**5.1**	**798**	**261**	**296**	**739**	**2094**	**229**	**406**	**1459**	**1443**
27*	**720**	**5.8**	**281**	**245**	**316**	**407**	**1249**	**173**	**458**	**618**	**630**
28	600	5.8	190	231	242	17	680	120	401	142	114

Data obtained during periods of seizures are indicated by heavy-faced type.

* Large refusals of food. Except on these days the diet was completely taken.

† Specimen incomplete.

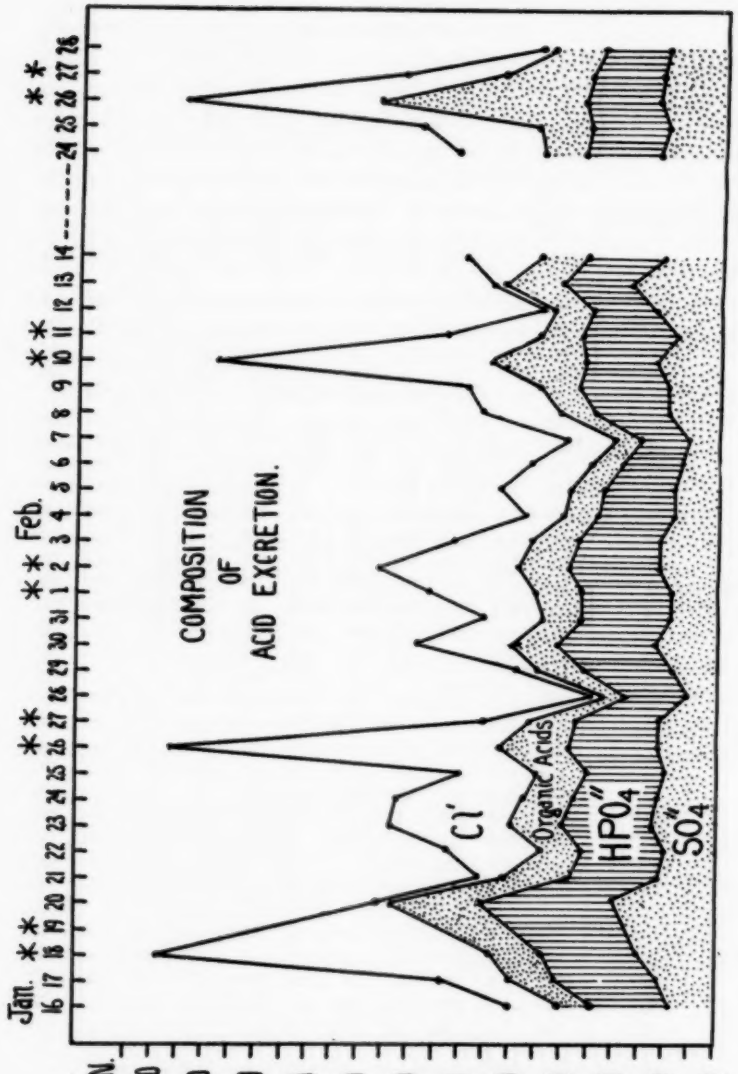
COMPOSITION
OF
ACID EXCRETION.
C.C.
0.1N.
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Jan.
16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
Feb.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 24 25 26 27 28
Cl'
Organic Acids
HPO4"
SO4"

FIG. 1-A

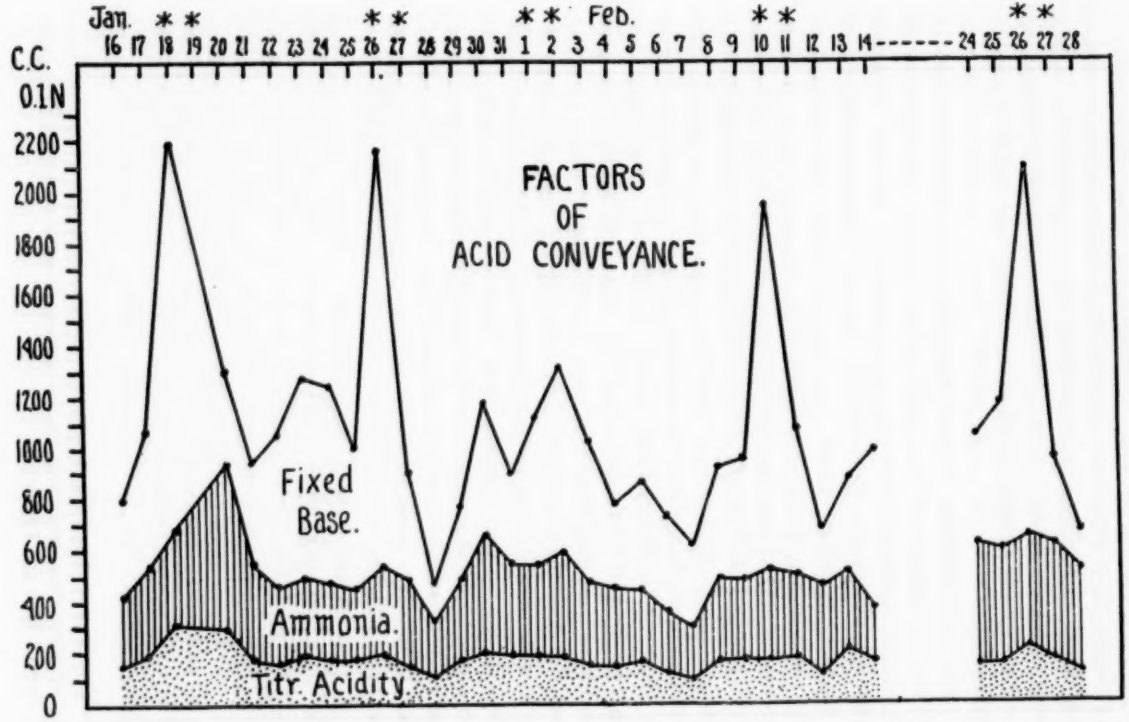
Jan.
Feb.
C.C.
0.1N
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
FACTORS
OF
ACID CONVEYANCE.
Fixed
Base.
Ammonia.
Titr. Acidity

FIG. 1-B

After an interval of ten days, the collection of urine was resumed and continued for five days during which time another group of seizures occurred. Only during the third of these five periods of active epilepsy were attacks of grand mal absent. Collection of urine was complete so far as could be determined except during the second day of the first period.

The measurements obtained from the urine specimens were the following: Volume, pH, titratable acidity (to pH 7.4), organic acids, sulphates (inorganic), phosphates (inorganic), chlorides, ammonia, and total fixed base. From the first thirteen specimens measurements of potassium and of calcium were also obtained. The values found for the acid-base factors are expressed in the tables as cc. of tenth-normal solutions; those for the radicals of the weak acids (the organic acids and phosphoric acid), which cover less base in urine than in blood plasma, are given in terms of their base equivalence at pH 7.4. The extent of base economy in the excretion of these radicals in acid urine is measured by its titratable acidity (to pH 7.4). This value, plus that for ammonia, subtracted from the sum of the acid radicals gives an indirect estimation of fixed base in the urine.[4] Total fixed base was also directly determined in the urine, by the method of Fiske (6), in order to obtain a more precise measurement of this important factor. Incidentally, abnormal organic base, if present, would be indicated by an excess of the indirectly over the directly obtained values for fixed base. In the tables the data obtained from the specimens collected during the days when epileptic seizures occurred are indicated by heavy-faced type.

As may be seen from the measurements in Table I, there occurred during the periods of seizures extensive change in several factors. The outstanding alterations are found in the values for total acid and for total fixed base. Both of these factors are very greatly and closely equivalently increased. The separate measurements of the four urine acids make it clearly evident that the large extension of total acid is chiefly due to an increased excretion of chloride ion. It is particularly significant that the two adjustable factors, titratable acidity and

[4] For a more detailed discussion of the process of acid conveyance into urine and of the method of study here used the reader is referred to Section V of a paper by Gamble, Ross and Tisdale (7).

ammonia production, which together regulate the use of fixed base in the process of acid excretion, are apparently undisturbed by the large losses of fixed base during the periods of active epilepsy. It may be noted here that immediately following these periods a recovery of the losses of fixed base and of acid, especially of chloride ion, is indicated by very low excretion values. These findings are more easily visible in the diagrams presented in Figures 1-A and 1-B which are built from the measurements in Table I. In Figure 1-A the composition of the total acid excretion is displayed by superimposing the values found for the four component factors. Here it is clearly evident that the

TABLE II

DAY	$K^{\cdot}$	$Ca^{\cdot\cdot}$	FIXED BASE $-$ ($K^{\cdot} + Ca^{\cdot\cdot}$)
	cc. 0.1N	*cc. 0.1N*	*cc. 0.1N*
January 16	213	24	122
17	258	37	234
18	**397**	**94**	**1113**
19			
20	164	49	108
21	178	37	174
22	281	18	295
23	380	26	390
24	355	24	393
25	299	33	229
26	**387**	**18**	**1293**
27	**217**	**20**	**511**
28	143	9	0

Data obtained during periods of seizures indicated by heavy-faced type.

large extensions of chloride ion excretion almost entirely explain the peaks for total acid. It may be noted, however, that the line in the diagram which measures the sum of the other three acid factors clearly indicates that they also share to an appreciable though relatively much less extent in the elevations of the total acid value. Curiously, during one of the groups of seizures (February 26–27) there was an increase of the organic acid excretion which was almost as large as that of chloride ion. The diagram in Figure 1-B is constructed by transferring from Figure 1-A the line defining the total acid excretion and by superimposing from the base line the values found for

titratable urine acidity (to pH 7.4) and for ammonia. The remainder of the distance to the total acid line represents the indirect measurement of fixed base. The diagram thus describes the factors in the conveyance of the acid radicals from the blood plasma into the urine and shows quite clearly that the titratable acidity and ammonia factors, which are greatly extensible in defence of fixed-base retention, here permit the excursions of total acid excretion to withdraw an approximate equivalence of fixed base. In Table II are given measurements of potassium and of calcium in the urine specimens from the first thirteen days of the period of study. By assuming that the magnesium factor in the composition of the total fixed base excretion in urine is relatively a very small one, we may take the remainder of total fixed base, after subtracting the sum of the values found for potassium and calcium, as an approximate measurement of sodium. From the data in this table it is learned that the large extensions of fixed base excretion are almost entirely composed of sodium.

The findings just presented indicate a change in body-fluid volume rather than in body-fluid reaction. The following considerations will serve to explain briefly this interpretation. It is evident that if the concentrations of substances in body fluids are to remain at normal values in the presence of a change in volume, adjustment by excretion or retention must occur, and to an extent which is defined for the individual substances by their relative magnitudes as factors in the composition of the body fluid concerned. Thus a reduction of the volume of intra-cellular water should, as regards excretion of electrolytes in the urine, produce an acid-base increment composed chiefly of potassium and of phosphate ions, since these are contained in cell fluid at relatively large concentrations. In the case of extra-cellular water, however, nine-tenths of the base present is sodium and quite two-thirds of the total base equivalence is covered by chloride ion. We may, therefore, expect that, in the presence of a considerable decrease in the volume of extra-cellular body fluid, the chief alterations in the acid-base composition of urine will consist in large additions to the excretion of sodium and of chloride ions. The excretion of the other acids and bases should also be increased but to only a relatively slight extent because of their very much smaller concentrations in this body fluid. It will also be apparent that a loss into the

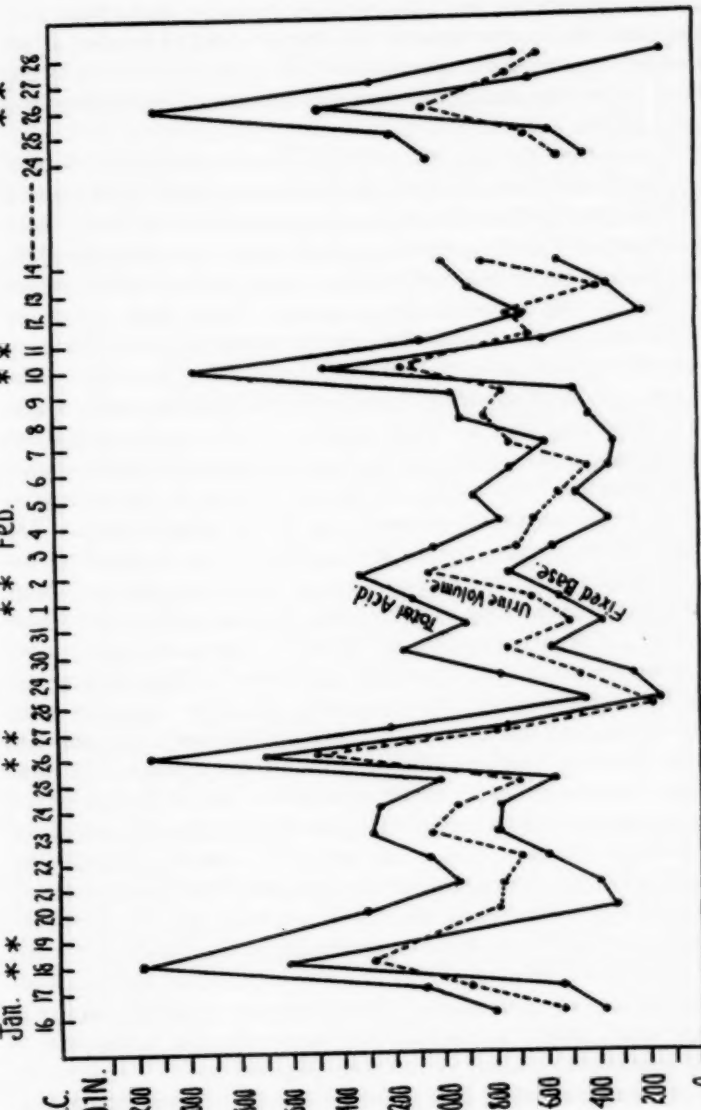
C.C. 0.1N.
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Jan. ** ** ** Feb. ** **
16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 1 2 3 4 5 6 7 8 9 10 11 12 13 14 24 25 26 27 28
Total Acid
Urine Volume
Fixed Base

FIG. 2

urine of a portion of a body fluid with its contained electrolytes is an event which in no way concerns the process of acid excretion as adjusted for a given diet. Alteration of the regulatory factors in this process, titratable acidity and ammonia production, is therefore not to be expected.

The data from the epileptic subject of this study describe changes in the acid-base composition of the urine which agree in all respects with those just postulated for a loss of extracellular body fluid. During the period of study a stationary fluid intake was maintained, the patient was kept in bed, and physical environmental circumstances were day by day approximately constant. Under these conditions any considerable loss of body fluid should produce an at least roughly corresponding increase in the volume of urine. This was found to be the case. In the diagram in Figure 2, the measurements of total acid and of total fixed base are given together. Here the determinations of fixed base by the direct method are used because of their probably better accuracy. The closely parallel relationship of the extensions of these values suggesting a loss of body fluid is clearly shown. The measurements of urine volume are recorded in the diagram by the points connected by the broken line and it may be seen that large increments corresponding with the peaks for total acid and fixed base occurred. The measurements are plotted in cubic centimeters using the scale for cc. 0.1 N. That these additions to urine volume are actually of a size to suit the conception that they represent body water accompanying the increased excretion of electrolytes is indicated by the following rough calculation. Taking the average daily urine volume, which was 746 cc. for the twenty-nine days of the first period of study, as the correct level of water excretion in urine; the additional excretion for January 18th, a day of severe seizures, was 1280–746, equalling 534 cc. Using similarly the average for fixed base excretion, 587 cc. 0.1 N, the increased excretion on the same day was 1604–587, equalling 1017 cc. 0.1 N. If the concentration of fixed base in the water of blood plasma, which is 1.72 cc. 0.1 N be regarded as approximately representative of extra-cellular body water; the calculated volume of water which should accompany the just estimated withdrawal of fixed base is 1017 divided by 1.72, equalling 592 cc.

Regarding, then, these data as indicating a loss of extra-cellular body

fluid during the periods of seizures, the question at once presents itself: Is this event referable to an antecedent instability of regulation of volume within the body which may be taken as of a primary pathogenic significance, or is it simply an incidental product of the activity of disease factors? These data unfortunately do not inform us on this critically interesting point. Of possible significance here it may be mentioned that during the fasting state, which is relatively refractory to an activity of epilepsy, a reduction of the volume of body fluids, especially of extra-cellular water, occurs (7). It is also well known that ingestion of acid-producing salts tends to cause this change.

SUMMARY

The acid-base composition of urine was determined in a series of 24-hour specimens from an epileptic child. An accurate constancy of dietary factors and of fluid intake was maintained. It was found that during periods of seizures a greatly increased excretion of total acid and of total fixed base occurred. The increment of total acid was chiefly composed of chloride ion, while the approximately corresponding extension of fixed base was nearly entirely sodium. The factors regulating the excretion of fixed base, viz. urine acidity and ammonia, remained nearly stationary. These findings are taken as indicating a loss of extra-cellular body fluid in the urine during the periods of active epilepsy. Agreeing with this interpretation, corresponding increments of urine volume were found.

REFERENCES

(1) GEYELIN, H. R., BIGWOOD, E. J., AND WHEATLEY, M. A.: *Proc. Soc. for Exp. Biol. and Med.*, 1923–24, xxi, 227.
(2) BIGWOOD, E. J.: *Ann. de médecine*, 1924, xv, 24.
(3) LENNOX, W. G.: *Proc. Am. Soc. for Clin. Invest.*, 1927; *Jour. of Clin. Invest.* (in press).
(4) BISGAARD, A., AND NOERVIG, J.: *Compt. rend. des séances Soc. biol.*, 1921, lxxxiv, 318.
(5) HASSELBACH: *Biochem. Ztschr.*, 1916, lxxiv, 18.
(6) BIGWOOD, E. J.: *Bruxelles méd. rev.*, 1923, i, 1.
(7) GAMBLE, J. L., ROSS, G. S., AND TISDALE, F. F.: *J. Biol. Chem.*, 1923, lvii, 633.
(8) FISKE, C. H.: *J. Biol. Chem.*, 1922, li, 55.

THE METABOLISM FOLLOWING INJECTION OF DIPHTHERIA TOXIN IN DOGS[1]

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This study was undertaken with a view to expanding some work done by Dr. Howland in 1909 (1) on the metabolism of patients suffering from diphtheria, in which he found evidence of widespread destruction of body protein. The principal findings were a prolonged negative nitrogen balance and at the same time a considerable excretion of creatin, both of which persisted long after the fever had subsided. The increased output of nitrogen was coincident with the appearance of albuminuria when the latter occurred, and it is of interest that, of the five patients having the most prolonged negative nitrogen balance, all had severe involvement of the pharynx, had remained untreated for four or more days, and developed myocarditis, in three of them well marked and in two moderate. The studies here recorded were made on dogs following the intravenous injection of diphtheria toxin in the hope that the above observations might be confirmed under more favorable experimental conditions.

The literature on the subject of metabolism following the injection of diphtheria toxin is not extensive. In 1895 a group of French investigators studied the effects of large doses of the toxin on dogs. Courmont and Doyon (2) noted a rise in temperature lasting not less than fifteen hours followed by a rapid fall ending in death. The rectal temperature reached a point between 25° and 30°C. They found that by keeping the dogs in a cold environment the hypothermia became more marked. One dog that they injected with a smaller amount of toxin survived 2 months, became paralyzed after about fifteen days, and lost considerable weight. His temperature fell about one degree after a short period of latency and remained lower than normal for five or six days. They called attention to the vaso-dilator

[1] Received for publication September 20, 1927.

action of the toxin and believed that it was responsible for the downward course of the temperature.

Arloing and Laulanié (3) studied the respiratory exchange after the injection of large doses of toxin. They found a rise in the utilization of oxygen during the period of fever, followed by a marked fall as the temperature became lower. This fall in oxygen utilization was accompanied by a lowered respiratory quotient.

De Croly (4) in 1898 studied the effect of diphtheria toxin on the metabolism of rabbits, including a study of the excretion of total nitrogen. The urine specimens were quite irregular, but there can be no doubt that he obtained a rise in nitrogen output after a period of latency in which the nitrogen excretion fell below normal. This rise was coincident with a more rapid fall in weight than had occurred up to that time, and in one rabbit was also coincident with albuminuria and diarrhea. The day before death the quantity of nitrogen in the urine decreased. In the more severe intoxications there was a slight rise in nitrogen excretion on the first day, followed by a very rapid drop until death took place on the third or fourth day. No temperature records were made.

In the study reported in this paper the method of attack was fairly uniform throughout. Each dog was studied through a fore-period to determine the normal limits of variation of the nitrogen excretion, temperature, etc. This period in a number of instances included a short study of the effect of starvation. The toxin was then injected intravenously in one dose, and the dog was again studied until she died, or until it was thought she had become normal again, or else had developed complications that rendered further study without profit.

Dose of toxin. This was varied, but the best results were obtained with a dose between 0.6 and 0.7 m. l. d. per kilogram of body-weight. The narrow limits of the optimal dose, the variability in the reaction of the dogs, and in one or two instances the deterioration of the toxin were sources of difficulty.

Food. The dogs were fed on white bread and milk, or dog biscuit and milk, the food intake being kept constant throughout each experiment. The caloric intake was in all cases rather low. The protein intake was between 1.5 and 2.5 grams per kilogram of body-weight in most cases, but was higher in one or two. The food was well taken

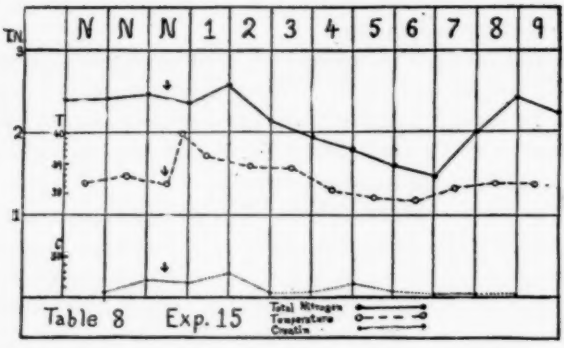

CHART 1

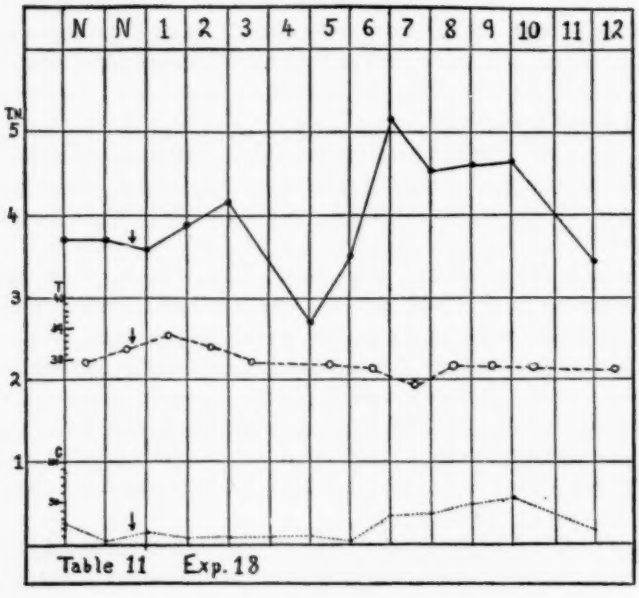

CHART 2

throughout, except in the more severe intoxications, and the stools were usually well formed.

Collection of specimens. The dogs were kept in a metabolism cage and twenty-four- or forty-eight-hour specimens collected, with catheterization to mark the end of each period. After each catheterization the bladder was washed with boric acid solution, to prevent cystitis.

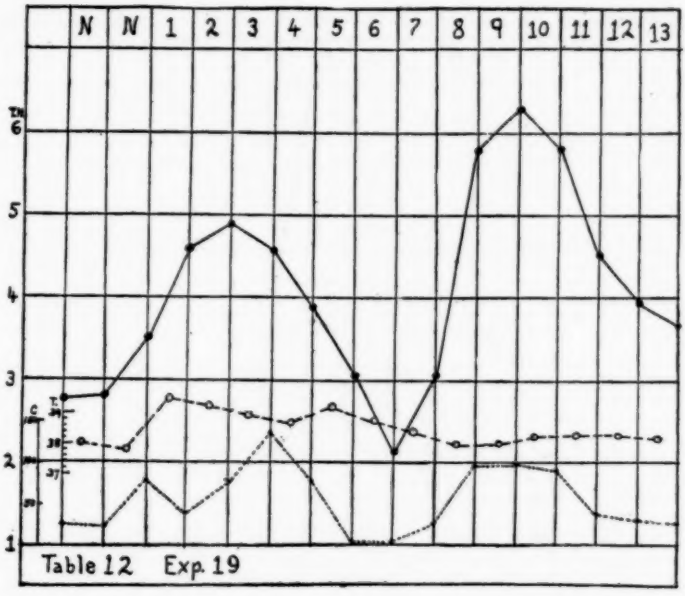

CHART 3

In the experiments in which the respiratory exchange was determined, the catheterization was usually omitted, because it was found that the association of catheterization with the other procedures rendered the dogs rather excitable so that it was difficult to obtain sufficient quiet. The total specimen of urine was mixed and preserved with chloroform until such time as the determinations were to be made. The stools were disregarded.

The details of the collection of air for the determination of respira-

tory exchange are important. The determinations in the warm environment were always the first to be made in the early morning after an eighteen-hour fast. The dog was taken from her cage, carried down to the room where the determination was to be made and gently fastened to the table on her back or side with the abdomen fully exposed. The temperature of the room was warm, between 25° and 30°C., although good results were obtained from one dog with the

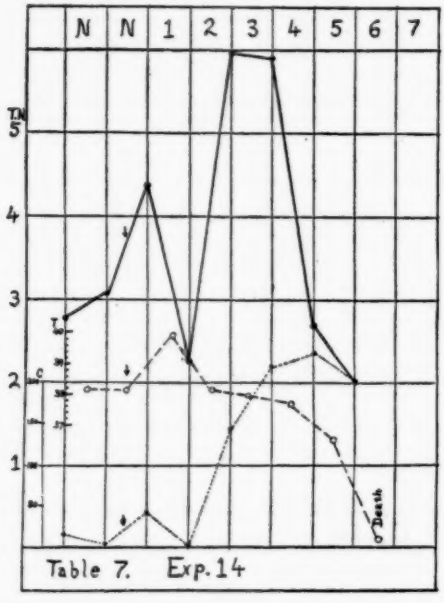

CHART 4

temperature as low as 23°C. All noise was as far as possible excluded and the air was collected before the other laboratory workers had arrived. A mask made from the smallest obtainable foot-ball bladder was used. Both ends were cut off, one end being slipped over the dog's nose and mouth, the other over a T-tube of wide gauge, fitted with Tissot valves. After the dog had been allowed to breathe through the apparatus for about five minutes, the collection was made over a

period of from 3 to 5 minutes, the air being collected in a rubber bag of about eighteen litres' capacity. Immediately on termination of the period of collection, a 30 cc. sample of air was removed for analysis, and the collection apparatus tested for leakage, by stopping the outlet. The volume of air was measured in a small spirometer and the sample analyzed in a Haldane gas analysis apparatus. The dogs lay very quietly after they had become used to the procedure so that good values for resting metabolism, if not basal metabolism, were obtained.

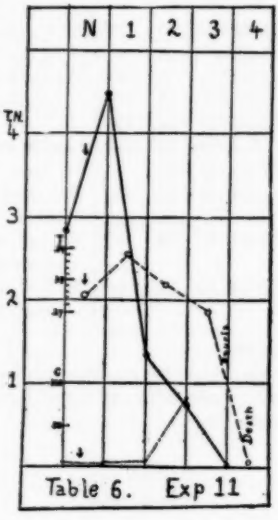

CHART 5

While the figures for the respiratory quotient cannot be relied on to any great extent, I feel justified in using them in drawing certain conclusions which will be discussed below.

The results of these experiments are contained in the charts and tables that follow. The moderate or moderately severe intoxications offer the best choice for study and it is on the results of these that the discussion will be based. (Tables I, II, III, IV, V, VIII, X, XI, XII, XIII; Charts 2, 3, 4.) The course was fairly regular. Within a few

TABLE I

Experiment 1

Experiment begun 19/XI/20.
Diet: 200 cc. milk, 50 grams bread.
Food intake: Protein, 12 grams; carbohydrate, 34 grams; fat, 9 grams.

DATE	DAY	WEIGHT	TEMPERATURE	URINE					REMARKS
				Volume	Total nitrogen	Creatinin	Creatin	Albumin	
		kg.	*°C.*	*cc.*	*gm.*	*mg.*	*mg.*		
20/XI	N	4.8	38.4	160	1.42	110	0	Tr.	
22/XI	N	4.7	39.2	330	1.62	133	0	0	
23/XI	N	—	38.6	310	1.40	103	8	0	Toxin 1.2 m.l.d. per kilogram
24/XI	1	4.7	40.3	365	1.37	113	5	Tr.	
25/XI	2	4.6	38.6	165	2.00	140	14	Tr.	
26/XI	3	4.4	38.0	90	*—	96	6	—	Loss of appetite. Diarrhea
27/XI	4	4.3	38.0	220	2.64	124	35	+	No food taken. Jaundice
28/XI	5	4.2	39.8	170	2.84	99	83	+	No food taken. Paralysis first noted
29/XI	6	4.1	37.9	118	1.32	59	89	+	No food taken. Tremor. Rapidly progressing paralysis
30/XI	7	3.9	37.2	90	0.53	36	97	+	No food taken. Convulsions. Vomiting

Dog was found dead 1/XII/20.

* This urine specimen was spoiled for nitrogen determination because of the presence of blood. On the basis of the other experiments and of the oliguria, it is probable the total nitrogen excretion on this day was low.

TABLE II

Experiment 2

Experiment begun 7/XII/20.
Diet: 350 cc. milk, 80 grams bread.
Food intake: Protein, 20 grams; carbohydrate, 54 grams, fat, 15 grams.

DATE	DAY	WEIGHT	TEMPERATURE	URINE					REMARKS
				Volume	Total nitrogen	Creatinin	Creatin	Albumin	
		kg.	*°C.*	*cc.*	*gm.*	*mg.*	*mg.*		
8/XII	N	7.8	38.8	270	3.04	213		Tr.	
9/XII	N	7.7	39.0	260	2.93	221		—	Toxin 0.63 m.l.d. per kilogram.
10/XII	1		39.7	280	3.44	226		0	

TABLE II—*Continued*

DATE	DAY	WEIGHT	TEMPERATURE	URINE					REMARKS
				Volume	Total nitrogen	Creatinin	Creatin	Albumin	
		kg.	*°C.*	*cc.*	*gm.*	*mg.*	*mg.*		
11/XII	2	7.8	38.7	225	3.34	209		Tr.	
13/XII	3, 4	7.8	38.3	375	2.95	210		—	
14/XII	5		38.7	210	2.49	195		—	
15/XII	6		38.1	250	2.84	215		Tr.	Somewhat less active
16/XII	7	7.8	38.1	350	4.29	213		+	
17/XII	8	7.7	38.3	395	4.23	201		+	
18/XII	9	7.6	38.1	340	4.22	220		+	
19/XII	10		38.3	410	3.73	198		+	
20/XII	11	7.3	37.7	400	4.23	226		Tr.	Weakness first noted
21/XII	12	7.3	38.2	360	4.28	219		Tr.	Fever and bloody nasal discharge. Distemper?
22/XII	13	7.2	39.3	280	4.32	224		Tr.	

The paralysis progressed rapidly and the dog died on the seventeenth day.

TABLE III

Experiment 3

Experiment begun 30/1/21.
Diet: 450 cc. milk, 100 grams bread.
Food intake: Protein, 26 grams, carbohydrate, 68 grams, fat, 19 grams.

DATE	DAY	WEIGHT	TEMPERATURE	URINE					REMARKS
				Volume	Total nitrogen	Creatinin	Creatin	Albumin	
		kg.	*°C.*	*cc.*	*gm.*	*mg.*	*mg.*		
1/II	N (2 days)	7.9	37.3	340	3.88	221	—	—	
3/II	N (2 days)	7.9	37.2	300	4.05	231	2	0	Toxin 0.63 m.l.d. per kilogram
4/II	1	—	38.4	285	4.21	235	29	—	
5/II	2	—	38.9	—	—	—	—	—	Miscarriage*
6/II	3	7.8	38.3	420	4.25	221	261	—	
7/II	4	7.8	38.3	195	4.15	232	19	0	Slightly less activity. Appetite good
8/II	5	—	38.9	370	4.20	223	1	Tr.	
9/II	6	—	38.3	260	2.70	225	19	+	Appetite poor (8–9/II). Ate about three-quarters of the food offered

* This experiment has been included in spite of the miscarriage because the creatin output on the third day indicates the involution of uterus took place then, so that the rest of the course was probably uninfluenced by it.

TABLE III—*Continued*

DATE	DAY	WEIGHT	TEMPERATURE	URINE					REMARKS
				Volume	Total nitrogen	Creatinin	Creatin	Albumin	
		kg.	°C.	*cc.*	*gm.*	*mg.*	*mg.*		
10/II	7	7.1	38.3	625	2.95	217	26	Tr.	Refused all her food (9–10/II)
11/II	8	7.1	37.2	575	4.70	232	16	+	Ate half (10–11/II)
12/II	9	—	37.8	675	7.18	243	36	—	Half diet
13/II	10	—	37.2	430	5.74	229	49	+	Half diet
14/II	11	6.8	38.1	460	5.61	244	6	—	Half diet
15/II	12	6.6	37.8	420	4.40	211	6	0	Half diet
16/II	13	—	—	410	3.94	223	—	—	Three-quarter diet. Weakness first noted

The paralysis and loss of weight progressed for about three weeks. The dog then became better and was normal again in two months.

Experiment 4. 0.4 m.l.d. of toxin per kilogram was found to be too small a dose to cause any changes in metabolism.

TABLE IV

Experiment 5

Experiment begun 2/III/21.
Diet: 400 cc. milk, 80 grams bread.
Food intake: Protein, 21 grams; carbohydrate, 56 grams; fat, 17 grams.

DATE	DAY	WEIGHT	TEMPERATURE	URINE					BLOOD		REMARKS
				Volume	Total nitrogen	Creatinin	Creatin	Albumin	N. P. N. per 100 cc.	Glucose	
		kg.	°C.	*cc.*	*gm.*				*mg.*	*per cent*	
3/III	N	8.2	38.1	340	3.10	220	—	0			
5/III	N (2 days)	8.2	38.0	360	3.15	224	—	—			
7/III	N (2 days)	8.2	38.1	315	2.97	227	0	Tr.	26.4		Toxin 0.8 m.l.d. per kilogram
8/III	1	8.2	38.6	540	4.16	223	16	Tr.			
9/III	2	—	38.2	200	3.91	231	0	Tr.			
10/III	3	—	38.2	290	3.78	223	5	Tr.	29.4	0.089	
11/III	4	8.2	38.3	230	2.26	228	15	Tr.			
12/III	5	—	38.0	255	2.10	230	20	Tr.			
13/III	6	8.4	37.6	475	3.19	230	5	—	44	0.092	
14/III	7	8.2	38.0	680	4.33	233	1	+			
15/III	8	8.2	38.1	450	4.65	235	12	—	33	0.089	
16/III	9	—	38.0	?		217	2	—			
17/III	10	8.0	37.8	315	3.29	234	—	0			

The dog remained well throughout the experiment and never developed any paralyses. This is the mildest intoxication that produced typical increase in the nitrogen excretion.

Experiment 6. The experiment was abandoned because the dog was given extra food by mistake from the fifth to the eighth day after the administration of the toxin. The dog was used two months later to test the development of immunity, after recovery from the partial paralysis caused by the toxin.

Experiment 7. Antitoxin was administered and three days later toxin 2 m.l.d. per kilogram were injected intravenously. No changes in metabolism followed.

TABLE V

Experiment 8

Experiment begun 20/IV/21.
Diet: 500 cc. milk, 40 grams bread.
Food intake: Protein, 23 grams; carbohydrate, 49 grams; fat, 21 grams.

DATE	DAY	WEIGHT	TEMPERATURE	URINE					BLOOD	REMARKS
				Volume	Total nitrogen	Creatinin	Creatin	Albumin	Non-protein nitrogen in 100 cc.	
		kg.	°C.	cc.	gm.	mg.	mg.		mg.	
21/IV	N	6.8	39.3	485	3.91	196	—	—		
23/IV	N	6.8	38.9	510	3.95	195	4	0	30	
25/IV	N	—	39.1	660	4.00	198	5	—		Toxin 1.2 m.l.d. per kilogram
26/IV	1	6.7	38.3	650	4.10	188	8	0	30.8	
27/IV	2	6.6	38.4	600	3.87	178	10	0	32	
28/IV	3	6.5	38.2	650	3.08	172	21	—	31.5	
29/IV	4	6.4	38.4	580	3.50	170	20	0	32	
30/IV	5	6.4	38.7	750	3.36	173	33	Tr.	32.5	First appearance of weakness. Slight muscular twitching
1/V	6	6.1	38.8	450	3.60	169	90	+	32.5	
2/V	7	5.9	38.9	500	3.98	164	85	+	34.5	Right-sided convulsions
3/V	8	5.4	37.6	480	3.76	160	171	+	—	Arterial pressure low. Marked paralysis. Convulsions

The paralysis became rapidly worse and the dog died later in the day (3/V). The temperature had dropped to 36°C. before death.

Experiment 9 (same dog as in Experiment 6). 0.4 m.l.d. of toxin per kilogram produced no result in the animal two months previously injected with 0.6 m.l.d. of toxin per kilogram.

Experiment 10. The toxin had become attenuated. The experiment was abandoned when it became evident that there would be no results worth recording.

hours after the toxin was injected the temperature rose and remained above normal for about 24 hours. The dogs ate well and remained lively. This was the first period, that of fever, and the metabolism was

about what one would have expected: a rise in the nitrogen excretion of longer or shorter duration accompanied by creatinuria, and sometimes, in the dogs so studied, a rise in oxygen consumption, which however was not very extensive (Table XIV). The next period was one of latency which lasted until the sixth or seventh day, during which the dog remained without symptoms. The temperature remained

TABLE VI

Experiment 11

Experiment begun 16/X/23.
Diet: 300 cc. milk, 30 grams dog biscuit.
Food intake: Protein, 18 grams; carbohydrate, 32 grams; fat, 13 grams.

DATE	DAY	WEIGHT	TEMPERATURE	URINE					REMARKS
				Volume	Total nitrogen	Creatinin	Creatin	Albumin	
		kg.	*°C.*	*cc.*	*gm.*	*mg.*	*mg.*		
17/X	N		37.7	175	3.19	251	0		
21/X	N	9.3	37.4	325	2.87	236	0		Toxin 0.8 m.l.d. per kilogram
22/X	1	9.15	39.0	420	4.55	280	0		
23/X	2	9.25	37.9	115	1.24	203	5	—	
24/X	3	9.3	37.0	145	0.74	94	75	+	Vomiting; tremor
25/X	4		33.3	0	0	0	0		No attempt to move. Paralysis? Complete anuria

The dog died about half an hour after the temperature of 33.3 was reached.

Experiment 12. The dog was given 1.1 m.l.d. toxin per kilogram and died in three days, with a temperature below 33°C. No urine was passed for twenty-four hours before death. The results did not differ to any extent from those in experiment 11.

Experiment 13. 0.8 m.l.d. toxin per kilogram produced death in five days. The clinical results were in every way similar to those of nos. 11 and 16. The urine specimens were too poor to make it worth while recording the results.

normal, and the appetite good. During this time the nitrogen excretion sank to normal or below, the creatin disappeared from the urine, and the general metabolism remained at about the normal basal level. Toward the end of this period there was at times a little tendency to refuse food and some listlessness, but usually the period of latency ended rather abruptly.

The third period, which might be termed that of intoxication, was

characterized by lessened appetite, lack of animation, slightly lowered temperature, sometimes diarrhea of short duration, and a tendency to bleed from mucous surfaces. These symptoms were usually lacking in the less severe intoxications, but in the more severe ones they became progressively worse, and in these death supervened in a few days. This period was marked by a rise in the excretion of nitrogen, which at times reached a very high level, a rise in creatin excretion and a lowering of the resting metabolism (Tables XIV, XV, XVI).

TABLE VII

Experiment 14

Experiment begun 17/XI/23.
Diet: 300 cc. milk, 30 grams dog biscuit.
Food intake: Protein, 18 grams; carbohydrate, 32 grams; fat, 13 grams.

DATE	DAY	WEIGHT	TEMPERATURE	URINE					REMARKS
				Volume	Total nitrogen	Creatinin	Creatin	Albumin	
		kg.	°C.	*cc.*	*gm.*	*mg.*	*mg.*		
19/XI	N	10.8	38.1	325	2.85	240	14		
20/XII	N	10.6	38.1	330	3.09	293	0		Toxin 0.7 m.l.d. per kilogram
21/XI	1	10.5	39.8	490	4.41	328	42		
22/XI	2	10.45	38.1	150	2.27	292	0	0	
23/XI	3	10.4	37.9	360	6.07	293	193	+	Dog is less active. Trembles considerably
24/XI	4	10.05	37.6	680	5.95	261	218	+	Jaundice. Vomiting
25/XI	5	9.6	36.4	630	2.72	185	235	+	Vomiting. Blood in stools. Paralysis beginning
26/XI	6	9.4	33.0	500	1.95	Very low	200	+	Died. Completely paralyzed

Albuminuria was regularly present during this period to the amount of about 0.5 to 1 gram per litre. The weight, which had been constant or had fallen only slightly up to this time, began to fall rapidly. In the more severe intoxications (Tables VI, IX, Chart 5) the rise in nitrogen excretion was only temporary, for as death became imminent the excretion became rapidly lessened, and during the last forty-eight hours might be very low. The creatin output roughly followed the degree of intoxication, but when the intoxication was very severe, there was a marked decrease in the excretion of urine and of the nitrogenous

TABLE VIII

Experiment 15

Experiment begun 6/XII/23.
Diet: 250 cc. milk, 25 grams dog biscuit.
Food intake: Protein, 15 grams; carbohydrate, 27 grams; fat, 11 grams.

DATE	DAY	WEIGHT	TEMPERATURE	URINE					REMARKS
				Volume	Total nitrogen	Creatinin	Creatin	Albumin	
		kg.	°C.	*cc.*	*gm.*	*mg.*	*mg.*		
8/XII	N (2 days)	6.9	38.4	295	2.41	195	0	0	
10/XII	N (1 day)	6.8	38.3	260	2.17	200	0	0	Toxin 0.5 m.l.d. per kilogram
11/XII	1	6.8	40.0	350	2.37	211	5	—	
12/XII	2	6.7	38.8	240	2.62	205	11	—	
13/XII	3	6.7	38.9	205	2.19	190	0	—	
14/XII	4	6.6	38.1	275	1.96	167	0	—	
15/XII	5	6.6	38.0	265	1.81	165	5	0	
16/XII	6	6.5	38.2	190	1.58	166	0	—	
17/XII	7	6.6	38.3	?	?	—	—	—	
18/XII	8	6.5	38.1	180	2.04	158	4	—	
19/XII	9	6.5	38.1	360	2.47	189	0	0	
20/XII	10	6.4	38.1	320	2.23	185	0	—	

The dog never showed the least evidence of intoxication.

TABLE IX

Experiment 16

Experiment begun 14/XII/23.
Diet: 350 cc. milk, 80 grams bread.
Food intake: Protein, 21 grams; carbohydrate, 54 grams; fat, 15 grams.

DATE	DAY	WEIGHT	TEMPERATURE	URINE					REMARKS
				Volume	Total nitrogen	Creatinin	Creatin	Albumin	
		kg.	°C.	*cc.*	*gm.*	*mg.*	*mg.*		
16/XII	N (2 days)	7.1	38.6	440	2.85	280	2	—	
17/XII	N	7.1	38.6	285	3.23	300	0	0	Toxin 0.67 m.l.d. per kilogram
18/XII	1	7.1	39.2	320	3.06	287	0	—	
19/XII	2	7.1	39.0	190	2.88	263	0	0	
20/XII	3	7.1	39.3	140	2.40	244	0	—	Activity markedly reduced. Diarrhea, vomiting
21/XII	4	7.1	38.3	80	1.01	159	65	+	Died. Completely paralyzed

TABLE X

Experiment 17

Experiment begun 23/II/24.
Diet: 470 cc. milk, 50 grams dog biscuit.
Food intake: Protein, 29 grams; carbohydrate, 54 grams; fat, 20 grams.

DATE	DAY	WEIGHT	TEMPERATURE	URINE					REMARKS
				Volume	Total nitrogen	Creatinin	Creatin	Albumin	
		kg.	°C.	*cc.*	*gm.*	*mg.*	*mg.*		
24/II	N	12.6	38.1	690	4.70	295	—		
26/II	N (2 days)	12.6	38.2	745	4.91	334	33		Toxin 0.6 m.l.d. per kilogram
27/II	1	12.5	39.8	470	4.95	365	16		
28/II	2	12.6	38.3	445	5.24	356	48		
29/II	3	12.6	38.2	740	7.90	379	137		
1/III	4	12.6	38.5	270	3.90	346	27	Tr.	
2/III	5	12.6	37.9	645	7.58	370	131	+	
3/III	6	12.4	37.8	595	6.48	222	49	+	Diarrhea
4/III	7	12.2	37.7	525	5.20	269	0	+	
5/III	8	11.9	37.7	590	6.27	340	20	+	
6/III	9	11.7	37.4	600	8.10	480	0		Fever in evening
7/III	10	11.7	37.9	375	—	—	—		Fever each evening

Paralysis first noted on 8/III when the dog was allowed to run around. It became progressively more severe and the dog died on the twenty-second day.

TABLE XI

Experiment 18

Experiment begun 9/III/24.
Diet: 450 cc. milk, 50 grams dog biscuit.
Food intake: Protein, 26 grams; carbohydrate, 52 grams; fat, 18 grams.

DATE	DAY	WEIGHT	TEMPERATURE	URINE					REMARKS
				Volume	Total nitrogen	Creatinin	Creatin	Albumin	
		kg.	°C.	*cc.*	*gm.*	*mg.*	*mg.*		
10/III	N	11.4	37.8	525	3.70	330	24		
12/III	N (2 days)	11.3	38.0	480	3.70	290	0		Toxin 0.6 m.l.d. per kilogram
13/III	1	11.3	38.4	460	3.55	300	12		
14/III	2	11.3	38.0	375	3.90	360	5	0	
15/III	3	11.3	37.8	625	4.15	350	10		
17/III	4 or 5	11.3	37.8	420	2.70	276	10	Tr.	
18/III	6	11.3	37.6	360	3.50	288	0	—	

TABLE XI—*Continued*

DATE	DAY	WEIGHT	TEMPERATURE	URINE					REMARKS
				Volume	Total nitrogen	Creatinin	Creatin	Albumin	
		kg.	°C.	*cc.*	*gm.*	*mg.*	*mg.*		
19/III	7	11.2	37.1	320	5.15	342	36	—	Somewhat less active
20/III	8	11.0	37.8	350	4.55	321	36	—	
21/III	9	10.8	37.7	400	4.63	330	50	—	
22/III	10	10.7	37.9	400	4.65	336	—		
24/III	11 or 12	10.6	37.7	310	3.45	330	—		First signs of beginning paralysis when dog runs about

The paralysis became gradually more severe and the dog died on the thirty-first day, completely paralyzed.

TABLE XII

Experiment 19

Experiment begun 14/V/24.

Diet: 470 cc. milk, 20 grams dog biscuit.

Food intake: Protein, 23 grams; carbohydrate, 30 grams; fat, 24 grams.

DATE	DAY	WEIGHT	TEMPERATURE	URINE					REMARKS
				Volume	Total nitrogen	Creatinin	Creatin	Albumin	
		kg.	°C.	*cc.*	*gm.*	*mg.*	*mg.*		
15/V	N	11.2	37.7	450	2.84	405	28	—	
17/V	N (2 days)	11.2	27.7	460	2.82	410	26	—	Toxin 0.7 m.l.d. per kilogram
18/V	1	—	39.2	700	3.45	442	88	—	
19/V	2	11.3	39.3	375	4.60	416	44	—	
20/V	3	11.3	38.5	375	4.85	392	80	0	
21/V	4		38.6	530	4.63	430	135	—	
22/V	5	11.25	39.2	370	3.89	410	80	—	
23/V	6		39.0	235	3.10	402	6	0	
24/V	7	11.2	38.1	175	2.16	175	6	0	Considerable urine lost. Values for nitrogen and creatin corrected on the basis of normal creatinin excretion
25/V	8	11.1	37.9	350	3.07	395	30	+	Slight diarrhea
26/V	9	10.7	37.8	725	5.80	458	96	+	
27/V	10	10.3	37.8	600	6.30	430	100	+	First signs of paralysis
28/V	11	10.1	38.1	380	5.71	426	90	Tr.	
29/V	12	—	38.7	330	4.50	402	35	—	
30/V	13	10.0	38.1	485	3.92	440	36	—	

The paralysis became progressively more severe and the dog was sacrificed on the nineteenth day when it was evident she would live but a few hours longer.

constituents. In such a case the creatin showed a sharp rise which, however, was only temporary and was followed by a sharp drop in the twenty-four hours preceding death (Table VI). As paralysis set in and rapidly progressed, the breathing became slower and finally ceased, apparently with involvement of the respiratory mechanism.

The dogs that survived this period showed another apparent return to normal. When they were allowed to run around, however, it was observed that they fell over rather easily. This was the beginning of the fourth period, that of paralysis, in which in fatal cases the dogs became progressively weaker until they finally died, usually at about the end of the third week, but once as late as the thirty-first day. The metabolism of this prolonged paralytic stage was studied in one instance, but the figures are not included in this paper. They show only what one might expect: metabolism within normal limits until the paralysis has become marked, shortly before death; then a rapid diminution in nitrogen excretion and oxygen comsumption, and as a result inability to maintain body temperature in a cold environment.

The circulatory apparatus was not studied by any special method. In one dog (no. 8) in which arterial puncture was done it was noted that twenty-four hours before death the blood pressure had fallen too low to force out the plunger of the syringe. There was no evidence of myocarditis clinically: the apex beat remained forceful, the first sound was of good quality, and there were no changes in the rate or rhythm of the pulse.

In the very severe intoxications (*e.g.*, Table IX) the changes in the condition of the dogs took place more rapidly and were correspondingly more marked. The premortal fall in nitrogen excretion took place immediately after the febrile rise. Vomiting, bloody diarrhea, hemorrhages in the skin and eye, jaundice, convulsions, and a progressive and marked fall in temperature were the outstanding features. The course was stormy and offered little opportunity for the study of metabolism. In the milder intoxications little happened except a slight drop in the nitrogen excretion during the period of "latency" (Table VIII; Chart 1).

DISCUSSION

1. Nitrogen excretion. The curve of nitrogen excretion confirms Dr. Howland's findings of 1909. There can be no doubt that the rise is due to the toxin. Fever does not account for it, for the dogs were afebrile during the period of the increased excretion of nitrogen. Its regular occurrence and the fact that it did not occur in a dog previously injected with antitoxin (no. 7) are sufficient evidence that the toxin was responsible for it. The normal values for non-protein nitrogen (Tables IV, V) would indicate that nitrogenous material was not stored and then suddenly liberated at the end of the latent period. There are two possible explanations for the rise in nitrogen: (*a*) oxidation of body protein to supply the needs of metabolism; (*b*) toxic destruction of body protein.

From a study of the respiratory exchange it does not appear that there was any difficulty in the oxidation of carbohydrate. The figures for respiratory quotient have been used to determine this point and I believe that they are quite good enough for the purpose (See especially Tables XIV and XV, seventh and eighth days). The conditions under which they were obtained were about the same as those under which the dog remained during most of the course of the experiment. In the case of fat, it appears at first sight that there might have been some difficulty in its oxidation during the height of the intoxication. A study of respiratory exchange in a cold environment, however, shows conclusively that fat could be used when needed,[2] so that the diminution in its oxidation that occurred when the dogs were at rest in a warm environment is probably due to the fact that protein and carbohydrate together supplied most of the needs of the resting metabolism. Moreover, it must be borne in mind that, to obtain values for the non-protein elements of the metabolism, I have assumed an even distribution of nitrogen excretion through the twenty-four hours, an assumption that would tend to make the calculated fat consumption lower than the actual since all determinations were made between

[2] The actual figures for the respiratory quotient on which this statement is based have not been included in the paper. They were for the most part quite low and from them I calculated that the extra calories expended by the dog as a result of a cold environment came in very large measure from the oxidation of fat.

TABLE XIII

Experiment 20

Experiment begun 30/V/24.
Diet: 200 cc. milk, 80 grams dog biscuit.
Food intake: Protein, 24 grams; carbohydrate, 65 grams; fat, 14 grams.

DATE	DAY	WEIGHT	TEMPERATURE	URINE					REMARKS
				Volume	Total nitrogen	Creatinin	Creatin	Albumin	
		kg.	*°C.*	*cc.*	*gm.*	*mg.*	*mg.*		
31/V	N	11.8	38.3		3.20	430	26		Toxin 0.72 m.l.d. per kilogram
1/VI	1	11.8	39.5		3.12	407	49		
2/VI	2		38.4		3.80	441	81		
3/VI	3	11.8	38.3		4.10	402	68		
4/VI	4	11.75	38.3		3.40	390	28	0	
5/VI	5	11.7	38.2		2.72	416	48	Tr.	
6/VI	6	11.5	37.9						Urine specimen entirely lost
7/VI	7	11.2	37.7		4.60	452	30	+	Appetite not so good. Ate three-fourths of the diet
8/VI	8		37.9		5.60	436	5	+	Poor appetite, two-thirds eaten
9/VI	9	10.7	38.2		4.90	416	30	+	No food eaten
10/VI	10	10.6	38.3		5.41	425	68	+	First signs of paralysis. Appetite improving
11/VI	11	10.6	37.9		4.98	424	75	+	About one-half diet eaten.
12/VI	12		38.2		5.20	423	66	+	One-half diet eaten
13/VI	13	10.2	39.0		3.08			+	Paralysis progressing rapidly. One-half diet eaten

After the thirteenth the dog refused to eat and her temperature rose considerably. She was sacrificed on the fourteenth day. At this time she was almost completely paralyzed but would probably have lived until the sixteenth or seventeenth day.

TABLE XIV

Experiment 17

Resting metabolism (determinations made in a warm environment while the dog was quiet)

DATE	DAY	ROOM TEMPERATURE	TEMPERATURE OF DOG	OXYGEN CONSUMPTION PER MINUTE	CALORIES PER HOUR	NON-PROTEIN R.Q.	REMARKS
		°C.	*°C.*				
25/II	N	30	38.2	85.0	24.8	0.75	Weight 12.6 kilogram
26/II	N	28	38.1	96.4	27.7	0.80	Toxin injected at 2 p.m.
27/II	1	27	39.4	110.3	32.7	0.93	
28/II	2	28	38.2	89.7	26.0	0.87	

TABLE XIV—*Continued*

DATE	DAY	ROOM TEMPERATURE	TEMPERATURE OF DOG	OXYGEN CONSUMPTION PER MINUTE	CALORIES PER HOUR	NON-PROTEIN R.Q.	REMARKS
		°C.	°C.				
29/II	3	29	37.9	92.5	26.7	0.80	Dog's temperature rose 0.2°C. in 10 minutes during determination
1/III	4	29	38.3	92.0	26.3	0.78	
2/III	5	30	37.8	95.0	27.4	0.81	Temperature rose 0.25°C. in 10 minutes during determination
3/III	6	30	37.4	88.3	25.4	0.79	
4/III	7	30	37.7	77.9	23.0	0.91	
5/III	8	30	37.7	71.0	21.1	0.93	

eighteen and twenty-four hours after the dogs had last been fed. From these considerations it is evident that the increased catabolism

TABLE XV

Experiment 18

Resting metabolism (determinations made in a warm environment while the dog was quiet)

DATE	DAY AFTER TOXIN ADMINISTRATION	ROOM TEMPERATURE	TEMPERATURE OF DOG	OXYGEN CONSUMPTION PER MINUTE	CALORIES PER HOUR	NON-PROTEIN R.Q.	REMARKS
		°C.	°C.	*cc.*			
13/III	1	26	38.3	98.7	28.8	0.85	
14/III	2	29	37.9	82.8	24.3	0.88	
15/III	3	26	37.6	88.1	25.6	0.86	Dog trembled slightly and temperature rose 0.1°C. during determination
17/III	5	29	37.8	79.0	23.2	0.89	
18/III	7	32	37.6	58.5	20.2	0.91	
20/III	8	29	37.8	68.4	20.1	0.90	
22/III	10	27	37.9	74.6	21.2	0.79	
24/III	12	28	37.7	71.7	20.8	0.83	
31/III	19	30	38.0	90.8	26.2	0.86	

of protein did not occur in order to supply calories which would be otherwise deficient as a result of failure of the organism to oxidize

TABLE XVI

Experiment 20

Resting metabolism (determinations made in a warm environment while the dog was quiet)

DATE	DAY AFTER TOXIN ADMINISTRATION	ROOM TEMPERATURE	TEMPERATURE OF DOG	OXYGEN CONSUMPTION PER MINUTE	CALORIES PER HOUR	NON-PROTEIN R.Q.	REMARKS
		°C.	°C.	cc.			
4/VI	4	25	38.3	86.1	24.8	0.80	
5/VI	5	23	38.2	83.8	24.0	0.80	
6/VI	6	24	37.9	82.5	23.8	0.82	
7/VI	7	24	37.7	69.6	20.0	0.81	
8/VI	8	26	37.9	66.6	19.2	0.81	
9/VI	9	23	38.1	68.3	19.8	0.80	Appetite very poor
10/VI	10	24	38.3	43.0	12.5	0.85	
11/VI	11	23	37.9	87.0	25.0	0.79	Slight trembling; 0.1°C. rise in temperature
12/VI	12	23	38.3	83.5	23.8	0.75	

TABLE XVII

To show the relation between the carbohydrate intake per kilogram and nitrogen output per kilogram at the height of the intoxication

NUMBER	CARBOHYDRATE INTAKE PER KILOGRAM	NITROGEN OUTPUT PER KILOGRAM		GREATEST RELATIVE RISE IN NITROGEN EXCRETION	REMARKS
		Normal	Highest in stage of intoxication		
	gm.	gm.	gm.		
1	0	0.29	0.67	2.30	Third day of starvation
19	3.1	0.26	0.61	2.32	
20	3.6	0.26	0.51	1.96	
3	4.2	0.50	1.05	2.10	
17	4.9	0.38	0.69	1.80	
18	4.9	0.32	0.48	1.50	
5	6.8	0.38	0.57	1.50	
2	7.1	0.38	0.55	1.43	
8	8.9	0.57	0.70*	1.23*	

* This apparent rise in nitrogen output does not appear in the chart or table for experiment 8. The value recorded here is nitrogen output per kilogram, and this dog was losing weight very rapidly.

TABLE XVIII

Experiment 17

Metabolism in a cold environment; to show inefficiency in the reaction to cold during period of intoxication

DAY AFTER TOXIN ADMINISTRATION	ROOM TEMPERATURE	CHANGE IN DOG'S TEMPERATURE	ACTIVITY (TREMBLING)	CALORIES PER HOUR	EFFICIENCY
	°C.	°C.			
N	17	+0.35	Slight	39.2	
3	15	+0.10	Slight	39.6	
4	16	0	None	35.4	No reduction
6	15	−0.10	Marked	42.9	Slight reduction
9	16	0	Marked	49.5	Marked reduction
4	20	0	None	31.2	
7	19	−0.20	None	30.0	Marked reduction
8	19	−0.15	None	30.8	Marked reduction

TABLE XIX

Experiment 18

Metabolism in a cold environment; to show inefficiency in the reaction to cold during period of intoxication

DAY AFTER TOXIN ADMINISTRATION	ROOM TEMPERATURE	CHANGE IN DOG'S TEMPERATURE	ACTIVITY (TREMBLING)	CALORIES PER HOUR	EFFICIENCY
	°C.	°C.			
2	15	+0.25	Slight	35.4	Normal
5	17	−0.15	Marked	42.1	Marked reduction
7	15	−0.15	Marked	39.6	Marked reduction
9	15	+0.10	Marked	36.3	Returning to normal
	15	+0.10		38.8	
10	15	+0.15	Slight	35.4	
N	19	+0.30		44.2	
2	19	0	Marked	35.4	
3	18	+0.15	Marked	43.0	No reduction
6	18	−0.10	Marked	42.5	Marked reduction
17	19	+0.30	Marked	43.0	No reduction

carbohydrate or fat. Nor did it occur as part of a general increase in the rate of metabolism, as the figures for oxygen consumption show. One is left then with the conclusion that the increase in nitrogen excretion was due to a specific toxic destruction of protein.

Determinations of urea and ammonia, made in a few instances, show nothing of importance. The ammonia excretion tended to rise shortly before death. Of more interest was the excretion of creatin, the extent of which paralleled fairly well the severity of the intoxication. This would seem to indicate that the increased nitrogen in the urine was derived mainly from the muscle tissue. The creatinin of the urine tended to decrease during periods of severe intoxication (Tables I, V, VI, VII, IX). This decrease may be connected with the destruction of muscle tissue. Further discussion of these points with the data that I have is hardly justified.

TABLE XX

Experiment 19

Metabolism in a cold environment; to show inefficiency in the reaction to cold during period of intoxication

DAY AFTER TOXIN ADMINISTRATION	ROOM TEMPERATURE	CHANGE IN DOG'S TEMPERATURE	ACTIVITY (TREMBLING)	CALORIES PER HOUR	EFFICIENCY
	°C.	°C.			
N	20	+0.50	Marked	37.1	
N	20	+0.25	Marked	39.6	
2	20	+0.10	None	31.3	
4	20	+0.30	Slight	32.9	
6	20	0	Slight	33.8	Only slightly reduced
8	20	0	Very marked	40.0	Markedly reduced
9	19	0	Very marked	42.2	Markedly reduced
13	20	+0.20	Very marked	38.7	Not reduced

2. Effect of carbohydrate. The effect of carbohydrate was not studied under very carefully controlled conditions. Such a study would require a larger series of cases because of the number of variables, some of them uncontrollable except through a statistical study—appetite, reaction to toxin, temperature, variation in the dose of the toxin, etc. After the figures had been gone over it was found that an effect of carbohydrate in reducing the nitrogen excretion could be demonstrated. This is shown in Table XVII. For the sake of comparison the nitrogen excretion and carbohydrate intake per kilogram of body-weight are used instead of the absolute values. Likewise, I have compared the relative rise of nitrogen excretion rather than

the actual rise in grams. This was obtained by taking the normal excretion of nitrogen per kilogram, *i.e.* the excretion per kilogram that was found in the period before toxin was given, and dividing this figure into that for nitrogen excretion per kilogram on the day on which this was greatest. The table shows clearly that the greater the carbohydrate intake the smaller the relative rise in nitrogen excretion. The results in the case of experiment 8 (Table V) were puzzling at the time they were obtained, for virtually no rise in nitrogen excretion was obtained, although such a rise was fully expected. From a consideration of Table XVII it may be seen that the high carbohydrate intake was probably responsible for the failure to obtain a rise in nitrogen excretion. I attempted in experiments 16 and 20 to repeat the high carbohydrate intake; in 16 there was too marked a reaction to the toxin and death followed too soon for results to be of any value, and in experiment 20 the dog refused to eat for a time. In experiment 19 the dog was given a low carbohydrate intake, and it may be seen that relative nitrogen excretion was the highest of any. From this study I am unable to say whether carbohydrate influenced in any way the pathological processes, prolonged life or saved life in a doubtful case.

The mechanism of the effect of carbohydrate on the nitrogen excretion was not studied and what figures I have throw little light on it. Carbohydrate did not affect creatin excretion to any appreciable extent. This negative result may be of some importance. During a fast, carbohydrate spares the use of body protein and with this sparing action goes a diminution of the creatin output. If creatin output means destruction of muscle protein, then the failure of carbohydrate to diminish creatin output means that carbohydrate has no influence on the toxic destruction of muscle protein, but that one must look elsewhere for its action. I believe this problem merits further study.

3. Temperature regulation. The tendency of the temperature to fall after the injection of diphtheria toxin was thoroughly demonstrated thirty years ago. The cause was ascribed by Courmont and Doyon to vaso-dilatation, and by Arloing and Laulanié to a marked lowering of the rate of metabolism as determined by oxygen consumption. The latter also noted that, in contradistinction to results in normal animals, the metabolism did not tend to rise in a cold room.

The results of a study of metabolism in a cold environment are contained in Tables XVIII, XIX, and XX. The procedure was the same as in the determination of respiratory exchange in the warm room, and all determinations were made before the dogs had been fed. In general the results show no failure to increase the metabolism in a cold room when such an increase is needed, but rather inefficiency in the reaction to cold during a period corresponding roughly to that of intoxication.

For the sake of clearness the data that best demonstrate the effect of a cold environment have been chosen for record in the tables. The other figures, if recorded, would render the tables rather confusing. The omitted figures are not contradictory, but are the ones for comparison with which I have no figures in a normal period.

This inefficiency in reaction to cold was shown by a tendency to a fall in the body temperature during the time the dogs were in a cold environment, even when the metabolic rate rose to a considerable extent, as contrasted with a tendency to a rise in the body temperature under similar conditions during periods of normal metabolism both before and after the period of intoxication. This may be illustrated by referring to the tables. In Table XVIII, during the normal period, which for this purpose extended through the fifth day after the administration of the toxin, it may be seen that, when the dog was placed in a room the temperature of which was about 16°C., her temperature tended to rise when the calories per hour rose to about 40, whereas on the sixth day with the same rise in metabolic rate there was a slight fall in body temperature. At a somewhat higher room temperature the same thing may be observed—at 30 calories per hour on the fourth day the dog maintained her body temperature, but was unable to do so on the seventh and eight days, although the metabolic rate was the same. Likewise in Table XIX, if one compares the fifth and seventh days with the second and ninth, one may see a tendency to falling body temperature even though the metabolic rate is higher than during the period of normal metabolism both before and after the period of intoxication. Again in Table XX, one should compare the eighth and ninth with other days before and after the intoxication period. The dogs trembled to a very marked extent in the effort to keep their temperature up to normal even when the temperature of the room was no lower than 22°C. (Dog 20.)

The dogs performed no external work during the period of collection of air, so that all the energy expended must have appeared as heat. This heat is either retained, causing a rise in body temperature, or else is dissipated by radiation or evaporation from the skin and lungs. If it may be assumed that changes in the rectal temperature measure equal changes throughout practically all the body (except the skin surfaces), then it must follow that for the same rise in rectal temperature, the same amount of heat has been retained, whatever the external conditions. The extra heat, the heat unaccounted for by a rise in temperature, is therefore dissipated either by increased radiation or evaporation of water. Calculations based on the humidity of the inspired air, and assuming complete saturation of the expired air, show that the extra heat could only in small part have been lost through the respiration. If diphtheria toxin has a vaso-dilator action, increased heat dissipation through the skin might well take place, and this may be, at least in large part, the explanation of the decrease in the efficiency with which the body temperature is maintained. The failure of Arloing and Laulanié to obtain a rise in heat production in a cold environment was undoubtedly due to the fact that they were dealing with a dog that was paralyzed in the last stages of intoxication and was therefore too weak to tremble.

SUMMARY

As one sums up what has been accomplished by this study three facts stand out. The first is the demonstration of toxic destruction of protein in an afebrile period. Whether this destruction of body protein contributed to the severe symptoms suffered by the dogs I cannot say from the results of these experiments. One may conjecture that the heart muscle has furnished its quota of the increased nitrogen excretion, but direct evidence of this is lacking, for weakness of the heart muscle was not a feature in the course of the intoxication, as it was in the case of children studied by Dr. Howland. It is probable that the increased urinary nitrogen comes largely from muscle tissue, and the lowering of resting metabolism during the intoxication period may merely be further evidence of this. The second fact is the prevention of the increased nitrogen excretion by a diet high in carbohydrate. The mechanism of this action is not clear. If carbohydrate

prevents the destruction of muscle protein, why does it not then diminish the excretion of creatin as it does when given during starvation? This effect of carbohydrate is deserving of further study, not only the effect on nitrogen excretion but also the effect on the actual pathological process and the course of the intoxication. The third fact is the demonstration of a period of latency, the duration of which bears an inverse ratio to the severity of the intoxication. Fourthly, the tendency to low temperature was studied and is ascribed to two influences. One is the vaso-dilator action of the toxin as a result of which more heat is lost from the skin surfaces than can be supplied by the rise in metabolic rate; the other, which is met shortly before death in severe intoxication and in the last hours of the paralytic stage, is the inability of the dog to maintain her metabolic rate at the ordinary level.

REFERENCES

(1) HOWLAND, JOHN: *Trans. Am. Paediat. Soc.*, 1909, xxi, 121.
(2) COURMONT ET DOYON: *Arch. de Phys.*, 1895, vii, 252.
(3) ARLOING ET LAULANIÉ: *Ibid.*, 1895, vii, 675.
(4) DE CROLY, O.: *Arch. Internat. de Pharmacodyn.*, 1898, iv, 385.

Articles not referred to in the paper

MINNE: *Arch. Internat. de Pharmacodyn.*, 1903, xii, 1. Study of the temperature and latent period in diphtheria intoxication.

KRASNOGORSKI: *Arch. f. exp. Path. u. Pharm.*, 1912, lxix, 239. Nitrogen excretion after relatively large doses of toxin.

MARENGHI: *Centrbl. f. Bakt.*, 1897, xxi, 256. Study of nitrogen excretion in horses being immunized with toxin. He obtained an increase in nitrogen excretion coincident with a rise in antitoxin values.

STUDIES UPON CALCIFICATION IN VITRO

II. On the Inhibitory Effect of the Magnesium Ion[1]

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It is now reasonably certain that the failure of calcification in rickets is due to an inadequate concentration of bone-forming elements in serum, namely, calcium ions, phosphate ions, or both. In infantile rickets, as well as in experimental rickets in rats, there is in most instances a marked deficiency of inorganic phosphorus in the serum. It is this deficiency which explains the failure of rachitic cartilage and osteoid to calcify both *in vitro* and *in vivo*. Shipley (1) failed to produce calcification in slabs of rachitic cartilage which were incubated in serum obtained from rachitic animals, but succeeded in obtaining calcification in the provisional zone of cartilage when similar sections of bone were placed in normal serum. Shipley, Kramer and Howland (2) repeated and confirmed Shipley's observation and showed in addition that the same results may be obtained with solutions of inorganic salts equivalent to serum freed from organic substances, provided that certain factors are adequately controlled, namely, the calcium and inorganic phosphorus concentrations and the reaction (pH) of the solution.

In a previous communication, we stressed the importance of the reaction of the solution in the process of calcification *in vitro* (3). We have found that the optimal pH for *in vitro* calcification is between 7.25 and 7.35. If the solution is too acid, calcification fails to occur; if it is too alkaline, precipitation instead of calcification takes place. In the present paper, we desire to report upon the marked inhibitory effect of magnesium ions upon calcification *in vitro*. We have found that calcification occurs most readily in the absence of mag-

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nesium. The introduction of magnesium ions, either in the form of magnesium chloride or magnesium sulphate, in excess of the normal

TABLE I

Effect of magnesium on calcification in vitro at pH 7.25 to 73.5 incubated at 37.5°C.

Ca	[Ca] $M \times 10^3$ per liter	P	[P] $M \times 10^3$ per liter	Ca × P	Mg.	[Mg.] $M \times 10^3$ per liter	Number of hours incubated	Number of sections incubated	Calcification*
mg. per cent		*mg. per cent*		*mg. per cent*	*mg. per cent*				
10	2.5	2.5	0.8	25	0	0	17–22	6	0
								5	+
10	2.5	2.5	0.8	25	1.8	0.75	18	12	0
10	2.5	3.0	0.96	30	0	0	17–22	2	+
								2	++
								11	+++
								4	++++
10	2.5	3.0	0.96	30	1.8	0.75	17–22	13	0
								1	+
								2	++
								1	+++
10	2.5	3.0	0.96	30	3.6	1.5	17–22	15	0
10	2.5	5.0	1.6	50	0	0	18–22	2	++++
								8	2 (++++)
								1	3 (++++)
10	2.5	5.0	1.6	50	1.8	0.75	18–22	9	++++
								3	2 (++++)
10	2.5	5.0	1.6	50	3.6	1.5	18–22	17	0
								4	+
10	2.5	5.0	1.6	50	7.2	3.0	17–22	9	0
10	2.5	6.0	1.9	60	3.6	1.5	17–22	12	0
10	2.5	7.0	2.2	70	3.6	1.5	17–22	2	+++
								2	++++
								2	2 (++++)
10	2.5	7.0	2.2	70	7.2	3.0	22	4	0

*++++ denotes calcification completely across the provisional zone of cartilage; + beginning calcification; ++ more advanced and +++ moderate or nearly a complete line of calcification. 2 (++++) and 3 (++++) designate calcification occurring also in primary and secondary tongues of cartilage.

concentration in serum, definitely inhibits the process, and the inhibitory effect progressively increases as the magnesium concentration rises. A concentration of 1.8 mg. of magnesium per 100 cc.

of solution or 0.75 mM. is capable of producing partial inhibition when the inorganic phosphorus concentration is 3.0 mg. per cent or 0.96 mM. Inhibition is complete when the magnesium concentration is doubled, that is, with 3.6 mg. per cent or 1.5 mM. However, as the inorganic phosphate concentration is increased, more magnesium must be added to bring about complete inhibition. The results are summarized in Table I.

PROCEDURE

The technic employed was essentially that used by Shipley, Kramer and Howland. The pH was determined colorimetrically and maintained between 7.25 and 7.35. The inorganic phosphate was varied from 2.5 to 7.0 mg. per cent or from 0.8 to 2.2 mM., expressed as phosphorus. The magnesium concentration was varied from 0.0 to 7.2 mg. per cent or from 0.0 to 3.0 mM., in multiples of 0.75 mM. or 1.8 mg. per cent. All the other components were kept approximately constant. The sections were incubated for from 17 to 22 hours at a temperature of 37.5°C. Aseptic technic was maintained throughout.

In every instance control experiments were carried out, a solution being employed in which calcification was found to occur regularly. The solution used for these controls had the following composition:

	mM.
Calcium	2.5
Phosphorus	1.6
Sodium	100.0
Potassium	5.7
Chlorine	75.0
Magnesium	0.75
Sulphur	0.75
Sodium bicarbonate	30.0

The reaction ranged from pH 7.25 to 7.35. The solutions were analyzed from time to time for all components and the agreement with the calculated concentrations was satisfactory.

DISCUSSION

Many hypotheses have been suggested to explain the failure of calcification in rickets. The recent studies of Shipley and of Shipley, Kramer and Howland have definitely established that this

failure is due not to an inability on the part of cartilage to take up lime salts but rather to an inadequate concentration of calcium or inorganic phosphorus, or both, in the medium surrounding these cells, or to an alteration in its reaction. This suggests that physico-chemical factors play a part in the process of calcification. The inhibitory effects of small amounts of magnesium ions reported here, and that of NaCl recently described by Shipley and Holt (4), lend further support to this hypothesis.

Holt, La Mer and Chown (5), as well as Hastings, Murray and Sendroy (6) have stressed the importance of the solubility products of $CaCO_3$ and $Ca_3(PO_4)_2$ in determining calcification, since these are the chief salts found in bone. They have calculated the so-called stoichiometric solubility product constants of these salts. Holt, La Mer and Chown have found $K'_{s.p.}$ $Ca_3(PO_4)_2$ to be much higher in serum as drawn than after saturation with solid $Ca_3(PO_4)_2$, so that normal serum is at least 200 per cent supersaturated as regards tertiary calcium phosphate. Holt (7) believes that even the serum of rachitic animals and infants is likewise supersaturated and suggests, in spite of negative histological evidence, that calcification is going on even in the rachitic organism. We have observed the cartilage cells in the callus of a fracture in a rat kept on a rickets-producing diet for 69 days, at the end of which time not a trace of calcification was to be seen,—a fact difficult to explain on the theory of supersaturation. Control animals fed on the same diet with which cod-liver oil had been incorporated calcified their calluses completely in fourteen days.

Hastings, Murray and Sendroy found the supersaturation hypothesis of Holt, La Mer and Chown to be inadequate to explain the data on solubility. Normal serum, when shaken with $CaCO_3$ did not lose calcium. When it was shaken with $Ca_3(PO_4)_2$, the loss of calcium was due not to the precipitation of $Ca_3(PO_4)_2$ but of $CaCO_3$. They assume that calcium exists in the serum, in part, as an unionized calcium compound similar in nature to calcium citrate. These authors have also calculated the solubility product of $Ca_3(PO_4)_2$ and obtained a value different from that of Holt, La Mer and Chown. Furthermore, their value for calcium-ion activity, as calculated from the stoichiometric solubility product of $Ca_3(PO_4)_2$, varies markedly from

that calculated from $K'_{s.p.}$ $CaCO_3$ in solutions with the same ionic strength as that of serum. It is obvious, therefore, that little is known regarding the concentrations of calcium and phosphate ions in serum, and of the activity solubility product of $Ca_3(PO_4)_2$. Until we have more definite data about these fundamental factors, no quantitative theory of calcification can be formulated.

Shipley and Holt (4) have recently demonstrated (and we have confirmed their observation) that an increase in the NaCl concentration of the solution above a certain level definitely inhibits the process of calcification *in vitro*. The addition of NaCl to a solution results, among other things, in raising its total ionic strength, thereby increasing the solubilities of $CaCO_3$ and $Ca_3(PO_4)_2$. The total ionic strength of serum is fairly constant and little is known regarding the variations in this quantity for tissue fluid. The increase of magnesium which suffices to inhibit calcification is too small to have a marked effect upon the total ionic strength of the solution. How then are we to explain the effect of the magnesium ion? Lewis and Randall (8), working with mixtures of different valence types, make the generalization that "in dilute solutions the activity coefficient of a given strong electrolyte is the same in all solutions of the same ionic strength." According to Bray (9) the activity coefficients of HCO_3^- in solutions containing $NaHCO_3$, Na_2CO_3 and NaCl are different from the values obtained when the solutions contain $KHCO_3$, K_2CO_3 and KCl. This would suggest that the nature of the cations in the solution is not a matter of indifference so far as the activity coefficient of any strong electrolyte is concerned.

In this connection the observations of Holt, La Mer and Chown are of interest. They have shown that the addition of 0.1 M or 100 mM $MgSO_4$ increased the solubility of $Ca_3(PO_4)_2$ 1660 per cent, while a similar amount of NaCl increased the solubility of this salt only 130 per cent. Thus it seems that the effect of the magnesium ion, in inhibiting calcification, can not be explained entirely on the basis of an increase of the total ionic strength, especially in the light of the fact that an inhibitory effect was obtained in our experiments with such minute amounts as 0.75 mM., but appears to be due to a specific effect of the divalent magnesium ion. It is possible that magnesium forms a slightly ionized phosphate compound, thereby reducing the

phosphate-ion concentration below the value for the solubility product of $Ca_3(PO_4)_2$. This seems plausible from the fact that the inhibitory effect of the magnesium ion can be overcome by suitable increments of inorganic phosphate.

CONCLUSIONS

Magnesium ions exert an inhibitory effect upon calcification *in vitro*. This becomes more marked with increasing concentrations of magnesium ions. The inhibitory effect of magnesium can be overcome by an adequate addition of inorganic phosphate to the solution.

The increase in magnesium which suffices to inhibit *in vitro* calcification might conceivably occur in the body fluids. It is so small as to exert a practically negligible effect on the total ionic strength of the solution.

REFERENCES

(1) SHIPLEY, P. G.: *Bull. Johns Hopkins Hosp.*, 1924, xxxv, 304.
(2) SHIPLEY, P. G., KRAMER, B., AND HOWLAND, J.: *Biochem. J.*, 1926, xx, No. 2, 379.
(3) KRAMER, B., SHELLING, D. H., AND ORENT, E. R.: *Proc. Soc. Exp. Biol. and Med.*, 1926, xxiv, 240.
(4) SHIPLEY, P. G., AND HOLT, L. E., JR.: *Bull. Johns Hopkins Hosp.*, 1927, xl, 1.
(5) HOLT, L. E., JR., LA MER, V. K., AND CHOWN, H. B.: *J. Biol. Chem.*, 1925, lxiv, 509.
(6) HASTINGS, A. B., MURRAY, C. D., AND SENDROY, J., JR.: *J. Biol. Chem.*, 1927, lxxi, 723, 783 and 797.
(7) HOLT, L. E., JR.: *J. Biol. Chem.*, 1925, lxiv, 579.
(8) LEWIS, G. N., AND RANDALL, M.: Thermodynamics and the Free Energy of Chemical Substances, New York and London, 1923.
(9) BRAY, W. C.: Dissertation, Yale University, New Haven, 1925.

THE EFFECT OF SUNSHINE ON THE ACIDITY OF THE INTESTINAL TRACT OF RACHITIC RATS[1]

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In 1923 Zucker and Matzner (1) showed that the administration of cod-liver oil to rachitic rats, or the exposure of the animal to ultra-violet rays produced a definite increase in the acidity of the faeces. The effect of cod-liver oil on the reaction of the small intestine of rachitic rats was studied in 1925 by Abrahamson and Miller (2). These investigators showed that both the upper and lower halves of the small intestines of normal rats had an acidity which varied from pH 5.2 to 6.3. On the other hand, the small intestines of 4 rachitic rats were much more alkaline, ranging from pH 6.4 to 7.0. The addition of cod-liver oil to the diet of similar rachitic rats brought the intestinal acidity to within the range of that of the normal animal. Jephcott and Bacharach (3) carefully studied the effect on the acidity of the faeces of rachitic rats of the administration of the antirachitic vitamine, or the direct irradiation of the animals, and confirmed the previous observations of Zucker and Matzner. Grayzel and Miller in 1927 (4) showed that both ultra-violet irradiation and the administration of cod-liver oil caused the alkaline intestinal reaction of rachitic dogs to return to the acidity found with the normal animal.

Zucker and Matzner (1) in their original investigations found that the exposure of rachitic rats to an amount of sunshine which tended to prevent the disease resulted in an increase in the acidity of the faeces. We have been unable to find in the literature any more work on the effect of sunshine on the acidity of the contents of the intestinal tract of rachitic animals.

During the past year an investigation of the seasonal variation of the antirachitic effect of sunshine has been carried out in this labora-

[1] Received for publication, July 20, 1927.

TABLE I

Hydrogen-ion concentration of the intestinal contents of normal rats

DATE (1927)	UPPER HALF OF SMALL INTESTINE	LOWER HALF OF THE SMALL INTESTINE	CAECUM	CAECUM AND COLON MIXED	COLON	FAECES
	pH	*pH*	*pH*	*pH*	*pH*	*pH*
June 20	6.5	6.6		5.8		
	6.4	6.0		6.3		6.4
	6.6	6.6		6.2		
June 27	6.2	6.1		5.4		
	6.4	6.3		6.0		6.2
July 11	6.2	6.1		6.0		7.3
	6.1	5.9		6.2		6.8
	6.0	5.8	5.9		6.6	7.2
	6.6	6.7	6.2		6.9	7.5
Average	6.33	6.23	6.05	5.98	6.75	6.90

TABLE II

Hydrogen-ion concentration of the intestinal contents of rachitic rats

DATE (1927)	UPPER HALF OF SMALL INTESTINE	LOWER HALF OF SMALL INTESTINE	CAECUM	CAECUM AND COLON MIXED	COLON	FAECES
	pH	*pH*	*pH*	*pH*	*pH*	*pH*
June 20	6.5	6.7		6.8		
	6.6	6.9		7.1		7.2
July 4	6.5	6.9		7.0		7.6
	6.6	6.6		6.9		7.7
	6.2	6.2		6.6		7.1
July 11	6.3	6.2	6.8		6.8	7.4
	6.4	6.5	6.8		7.1	7.7
	6.7	6.6	6.5		6.6	7.4
Average	6.47	6.57	6.70	6.88	6.83	7.44

tory. The procedure consisted in placing albino rats, which were 25 to 27 days old and weighed about 40 grams, on McCollum's rickets-producing diet 3143 (5). Some were kept inside and others were

placed outside daily under the condition to be investigated. Control rats were fed on a normal diet. After 4 weeks the rats were killed by bleeding under ether anaesthesia and the degree of rickets determined. These rats furnished the material for the present investigation.

Hydrogen-ion determinations were made on the contents of different parts of the intestinal tract and on the faeces by the procedure outlined by Tisdall and Brown (6). The faeces which were passed during

TABLE III

Hydrogen-ion concentration of the intestinal contents of rats on the rickets—producing diet and exposed to sunshine daily

DATE (1927)	DAILY DURATION OF EXPOSURE	UPPER HALF OF SMALL INTESTINE	LOWER HALF OF SMALL INTESTINE	CAECUM	CAECUM AND COLON MIXED	COLON	FAECES
		pH	*pH*	*pH*	*pH*	*pH*	*pH*
July 4	5 minutes	6.7	6.6		6.4		7.2
July 11	5 minutes	6.5	6.4	6.4		6.4	6.8
June 27	10 minutes	6.5	6.7		6.0		
	10 minutes	6.7	6.7		6.0		6.6
	10 minutes	6.4	6.4		6.0		
July 11	10 minutes	6.4	6.4	6.3		6.4	6.8
June 27	15 minutes	6.8	6.6		6.0		6.6
	1 hour	6.5	6.5		6.0		
	1 hour	6.5	6.5		6.0		6.4
	1 hour	6.7	6.6		6.0		
June 20	2 hours	7.0	6.6		6.4		
	2 hours	6.2	6.3		6.3		6.1
June 27	2 hours	6.6	6.6		6.2		7.3
July 4	2 hours	6.5	6.4		5.8		6.8
July 11	2 hours	6.6	5.9	6.1		6.0	6.6
Average		6.57	6.48	6.26	6.09	6.26	6.72

the administration of the anaesthetic were collected for these determinations. The intestinal tract of the rat was then dissected out and divided into 3 or 4 parts, the upper half of the small intestine, the lower half of the small intestine, the caecum, the colon or the caecum and colon together. The contents of the respective parts were gently expressed and the acidity determined. The determinations were made as rapidly as possible and all material not actually in use was kept in the ice-chest. The results obtained are given in the accompanying tables.

COMMENT

Our results confirm the observation of Zucker and Matzner (1) that exposure of rachitic rats to sunshine results in an increase in the acidity of the faeces.

From a survey of the tables it is seen that the acidity of the upper part of the small intestine of the normal rat is slightly greater than that of the rat on the rickets-producing diet whether exposed to sunshine or not. The intestine of the normal rat becomes progressively more acid. On the other hand, the intestine of the rachitic rat becomes more alkaline throughout its entire course. The acidity of the intestine of the rat on the rickets-producing diet, but exposed to sunshine daily, closely approximates that of the normal animal.

It is of interest to note that the acidity of the intestines of the rats exposed for only 5, 10 or 15 minutes daily is the same as that of the rats exposed for 1 and 2 hours. The antirachitic effect of the shorter exposures, however, is much less than that of the longer exposures. It is also of interest to note that the faeces of all the rats are much more alkaline than the contents of the large bowel. The importance of these results from the standpoint of the absorption and excretion of the inorganic elements by the intestines will be considered in another paper from this laboratory.

CONCLUSIONS

1. Exposure to sunshine of rats on McCollum's rickets-producing diet 3143 definitely increases the acidity of the lower half of the small intestine, the large intestine and the faeces.

2. The acidity of the intestines of these rats closely approximates that of the intestines of the normal animal.

3. Short daily exposures to sunshine produce just as marked changes in the acidity of the intestine as long exposures. These changes do not parallel the antirachitic effect.

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REFERENCES

(1) ZUCKER, T. F., AND M. J. MATZNER: *Proc. Soc. Exper. Biol. and Med.*, 1923, xxi, 186.
(2) ABRAHAMSON, E., AND E. G. MILLER, JR.: *Ibidem*, 1925, xxii, 438.
(3) JEPHCOTT, H., AND A. L. BACHARACH: *Biochem. J.*, 1926, xx, 1350.
(4) GRAYZEL, D. M., AND E. G. MILLER, JR.: *Proc. Soc. Exper. Biol. and Med.*, 1927, xxiv, 668.
(5) MCCOLLUM, E. V., SIMMONDS, N., SHIPLEY, P. G., AND E. A. PARK: *J. Biol. Chem.*, 1921, xlvii, 507.
(6) TISDALL, F. F., AND A. BROWN: *Amer. J. Dis. Child.*, 1924, xxvii, 312.

STUDIES ON CALCIFICATION IN VITRO

IV. The Effect of Inorganic Salts[1]

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Since Shipley (1) observed that slices of cartilage from a rachitic animal could be made to calcify outside the body in an apparently normal manner, a number of studies (2) have been carried out upon the factors which influence this calcification *in vitro*. Those which have been reported hitherto have been chiefly concerned with the effect of alterations of the reaction of the solution, the protein concentration, the concentrations of calcium and phosphorus and the effect of cell poisons. The inorganic composition of the artificial medium has usually been maintained at a close approximation to that of blood serum.[2]

It occurred to us that it might be of interest to study calcification *extra corpus* in a medium of somewhat different inorganic composition. Since many marine animals are able to lay down large amounts of calcium salts in their shell membranes the source of which is apparently the sea water, we were interested in seeing if sea water could furnish these salts to the tissue cells of warm-blooded vertebrates. Moreover, should this prove to be the case, we felt that it would be extremely interesting to ascertain if the lime salts deposited from sea water would approximate in their chemical constitution that of the marine shell rather than the bone of higher animals. We consequently undertook some experiments with samples of sea water obtained from the surface in the vicinity of Woods Hole, Massachusetts, during the early part of June, 1926. This sea water had an ion product[3]

[1] Received for publication, November 1, 1927.

[2] In the experiments of Shipley, Kramer and Howland (ref. no. 3) a somewhat lower NaCl concentration was used—the concentration of Na being about 43 mM and of Cl about 13 mM.

[3] Calculated as in the paper of Holt, LaMer and Chown. Ref. no. 3.

$[Ca^{++}]^3 \times [PO_4^{\equiv}]^2$ of about $10^{-25.7}$, but it differed particularly in its organic composition from blood serum in two other respects: in having a somewhat higher concentration of sodium chloride, and in having a considerably higher magnesium content.[4] In spite of repeated trials, we were unable to find any evidence of calcification in any of our preparations with this medium. Apart from the low ion product this result suggested that either the high salinity or the high magnesium content might be inhibiting this process.

That the concentration of inorganic salts other than those of Ca and P should exert a profound influence on calcification might well be expected. Holt, LaMer and Chown (3) recently observed the striking effects of various salts on the solubility of tertiary calcium phosphate, and showed by their calculations that their results[5] were in reasonable conformity with the Debye-Hückel theory. It remained to be shown, however, whether inorganic salts would influence the calcification of a living tissue. If this should be the case, would they only influence calcification as they might affect the solubility of $Ca_3(PO_4)_2$, or would there be, in addition, specific biological salt effects concerned, such as are known in heart muscle, in nervous and many other living tissues. In order to elucidate these questions we undertook a quantitative study of the effect of salts on the process of calcification *in vitro*.

The technique employed in these experiments was that described by Shipley, Kramer and Howland (2c) with certain exceptions which are noted below. Our observations on the presence of calcification in preparations were made after an incubation period of 20 to 24 hours' duration.

In our earliest studies we used a basal solution (solution A) of the following composition:

[4] The composition of the sea water used was approximately as follows:

Na	383	mM
K	10.5	mM
Ca	10.6	mM
Mg	53.3	mM
Cl	517	mM
P	0.02	mM
SO_4	28.3	mM

[5] Including those with magnesium salts.

Basal solution A

Na	34.1 mM
Ca	2.18 mM
Mg	0.8 mM
Cl	4.4 mM
P	1.33 mM
SO_4	0.8 mM
[6]CO_3	32.0 mM
Phenol Red	0.006 per cent

To this solution varying amounts of NaCl were added. The results of our experiments are given in Table I.

It is apparent that calcification was markedly inhibited when the Na content of the solution was increased to 76.9 mM, and that when

TABLE I

Effect of additions of NaCl on calcification in vitro

(Basal solution A)

NaCl added	total Na	total Cl	number of preparations	extent of calcification in 24 hours (average)
mM	mM	mM		
8.6	42.7	12.9	6	++++
21.4	55.5	25.7	3	+++
42.8	76.9	47.1	3	++
64.1	98.3	68.5	3	None
85.5	119.6	89.8	9	None

the amount of sodium was increased beyond this concentration, it was completely inhibited. Such a result is quite in harmony with the observations of Shipley, Kramer and Howland (2c) who obtained rapid calcification (within 24 hours) using salt solutions with a sodium content of about 43 mM. It is equally clear, however, that such rapid calcification will not occur when NaCl is present in concentrations as high as those of blood serum.

In extending our studies to include salts of different valence types: KCl, $NaHCO_3$, Na_2SO_4, $MgCl_2$ and $MgSO_4$, a somewhat different and

[6] The figure given for total CO_3 in this and subsequent experiments represents carbonate originally added as $NaHCO_3$, before the reaction was adjusted with CO_2.

simpler basal solution (solution B) was used which had the following composition:

Basal solution B

Na	11.9 mM
Ca	2.5 mM
Cl	5.0 mM
P	0.97 mM
CO_3	10.0 mM
Phenol Red	0.006 per cent

TABLE II

Effect of additions of NaCl on calcification in vitro

(Basal solution B)

NaCl ADDED	TOTAL Na	TOTAL Cl	NUMBER OF PREPARATIONS	CALCIFICATION
mM	*mM*	*mM*		
0	11.9	5.0	7	++++
8.5	20.4	13.5	6	++++
17.1	29.0	22.1	6	+++
23.8	35.7	28.8	3	+++
32.2	44.1	37.2	3	++
34.3	46.2	39.2	6	++
47.7	59.6	52.7	3	None
51.3	63.2	56.3	6	None
64.4	76.3	69.4	3	None

It will be seen that the concentration of sodium and chlorine which inhibited calcification, as shown by this table, differs slightly from that shown in Table I. This is to be explained by the difference in the basal solution in the two experiments.

For comparative purposes a new series of observations was made with additions of NaCl, using this new basal solution. The results of experiments with NaCl, KCl, $NaHCO_3$, Na_2SO_4, $MgCl_2$ and $MgSO_4$ additions are presented in Tables II and VII inclusive.

From the foregoing data, contained in Tables II to VII, it would seem that the following concentrations of added salts are sufficient to inhibit calcification under the conditions of these experiments.

NaCl	40–45 mM
KCl	50–55 mM
$NaHCO_3$	50–55 mM
Na_2SO_4	7– 8 mM
$MgCl_2$	less than 1 mM
$MgSO_4$	0.6– 0.7 mM

The marked effect of increase in the valence type of the salt studied is most conspicuous, and the magnitude of this effect suggests that

TABLE III

Effect of additions of KCl on calcification in vitro

(Basal solution B)

KCl ADDED	NUMBER OF PREPARATIONS	CALCIFICATION
mM		
0	6	++++
5.3	3	++++
10.7	3	++++
21.4	3	+++±
32.2	6	+++
47.7	3	±
64.4	3	None

TABLE IV

Effect of additions of $NaHCO_3$ on calcification in vitro

(Basal solution B)

$NaHCO_3$ ADDED	NUMBER OF PREPARATIONS	CALCIFICATION
mM		
0	3	++++
15	3	++++
40	3	++±
65	3	None
90	3	None

TABLE V

Effect of additions of Na_2SO_4 on calcification in vitro

(Basal solution B)

Na_2SO_4 ADDED	NUMBER OF PREPARATIONS	CALCIFICATION
mM		
0	3	++++
1	3	++++
3	3	+++±
6	3	±
10	3	None

calcification is a function of the ionic strength of the solution. The difference observed between the inhibitory action of NaCl and KCl, although small, appears to be a definite one, and is confirmed by the

experiments described below. The fact that Na^+ appears to inhibit calcification slightly more than K^+ does not, however, compel us to believe that we have here a specific biological effect of ions upon calcifying bone tissue. It will be recalled that Holt, LaMer and Chown[7] observed a slight difference in the solubility of $Ca_3(PO_4)_2$ in solutions of KCl and NaCl. The greater solubility was found in the case of NaCl. In other words Na^+, in inhibiting calcification, may be con-

TABLE VI

Effect of additions of $MgCl_2$ on calcification in vitro

(Basal solution B)

$MgCl_2$ ADDED	NUMBER OF PREPARATIONS	CALCIFICATION
mM		
0	3	++++
1	3	None
3	3	None
6	3	None
10	3	None

TABLE VII

Effect of additions of $MgSO_4$ on calcification in vitro

(Basal solution B)

$MgSO_4$ ADDED	NUMBER OF PREPARATIONS	CALCIFICATION
mM		
0	3	++++
0.16	3	++++
0.41	3	+++±
0.82	3	None
1.64	3	None

sidered to diminish the activity coefficient[8] of the salt $Ca_3(PO_4)_2$ somewhat more than does K^+.

The data on the inhibitory action of $MgCl_2$ cannot, however, be explained in this manner. If the inhibitory effect of this salt was a function of the ionic strength of the solution, one would expect that the

[7] Reference 3, pp. 538–544.

[8] A similar difference between the effects of K^+ and Na^+ on the solubility of other slightly soluble salts has been found by other observers.

effect of the di-univalent salt $MgCl_2$ would be approximately of the same magnitude as that of the uni-divalent salt Na_2SO_4. Holt, LaMer and Chown studied the solubility of $Ca_3(PO_4)_2$ in solutions of of $Mg(NO_3)_2$ and Na_2SO_4, and although their data on the former salt were incomplete it appeared reasonably certain that both salts exerted effects of the same order of magnitude, and of an order of magnitude quite different from that found with the di-divalent salt $MgSO_4$. The calcification experiments show, however, that $MgCl_2$ inhibits calcification to a far greater extent than Na_2SO_4. Such a result can only be explained on the basis of a specific biological effect of Mg^{++} upon the calcifying bone tissue.[9]

We have thus ample reasons for understanding the failure of our calcification experiments with sea water. In the first place, leaving aside the fact that the ion product $[Ca^{++}]^3 \times [PO_4^{\equiv}]^2$ is appreciably smaller in sea water than in blood serum, the ionic strength of the former medium is considerably greater than that of blood serum, and furthermore, we have a high concentration of an ion, Mg^{++}, which has a specific effect in inhibiting the deposition of tertiary calcium phosphate in living cartilage. Under these circumstances it is not difficult to comprehend why the calcareous deposits of marine animals are composed almost entirely of calcium carbonate and contain only traces of phosphate.

In continuing these experiments, it seemed desirable to use a basal solution of as simple composition as possible. The solution heretofore used had been prepared from $CaCl_2$ and Na_2HPO_4, hence Na^+ and Cl ions were always present. It was found possible to eliminate Na and Cl by preparing solutions from $Ca(OH)_2$ and H_3PO_4. Solutions which would permit calcification could be prepared from these two substances alone with the addition of a minimal amount of $NaHCO_3$ as buffer and the usual concentration of indicator. Great care was, however, necessary in the preparation of these solutions in order to prevent the deposition of secondary calcium phosphate,

[9] We have had the opportunity of seeing in press an article by Kramer and his associates which appears in this same issue. It is gratifying to note that these workers have, in an apparently quite independent manner, come to this same conclusion—namely, that the magnesium ion exerts a specific inhibitory influence upon calcification *in vitro*.

which, unlike the tertiary salt, is redissolved with great difficulty when the solution is acidified with CO_2. The following technique was found satisfactory. H_3PO_4 and $NaHCO_3$ were first dissolved in suitable quantities of water and CO_2 was then bubbled through the solution continuously, while small quantities of $Ca(OH)_2$ were introduced from time to time until the proper amount had been added. The solutions were kept acid with CO_2 until the time for their use, the reaction being then adjusted to 7.35 by allowing CO_2 to escape.

As might be expected because of their lower ionic strength, calcification occurred from such simple solutions with lower concentrations of calcium and phosphate. The results of such experiments are shown in Table VIII.

TABLE VIII

Calcification experiments with solutions prepared from $Ca(OH)_2$ and H_3PO_4

Na	CO_2	PHENOL RED	Ca	P	NUMBER OF PREPARATIONS	CALCIFICATION
mM	*mM*	*per cent*	*mM*	*mM*		
10	10	0.0006	2.67	0.970	3	++++
10	10	0.0006	2.67	0.728	3	++++
10	10	0.0006	2.00	0.485	3	±

We adopted the following composition for our new basal solution:

Basal solution C

Na	10.0 mM
CO_2	10.0 mM
Phenol Red	0.0006 per cent
Ca	2.5 mM
P	0.97 mM

It is evident from Table VIII that such a basal solution as the above contains concentrations of Ca and P *more than sufficient* to allow calcification to take place. It might, therefore, be expected that somewhat larger concentrations of salts would be required to inhibit calcification than in our previous experiments. This was found to be the case. Experiments were carried out to confirm the difference between the effect of NaCl and KCl and are presented below in Tables IX and X.

It can be seen that 70 mM of NaCl cause very marked inhibition of calcification, while this same concentration of KCl causes no inhibition of all.

TABLE IX

Effect of additions of NaCl on calcification in vitro

New basal solution (solution C)

NaCl ADDED	NUMBER OF PREPARATIONS	CALCIFICATION
mM		
0	3	++++
20	2	++++
30	3	++++
40	2	++++
50	5	+++
60	6	++±
70	5	+±

TABLE X

Effect of additions of KCl on calcification in vitro

(Basal solution C)

KCl ADDED	NUMBER OF PREPARATIONS	CALCIFICATION
mM		
0	3	++++
20	3	++++
30	2	++++
40	1	+++
50	5	++++
60	4	+++±
70	6	++++

CONCLUSIONS

1. The effect of various salts upon calcification has been studied by means of preparations *in vitro*.

2. Calcification of the intercellular substance of mammalian cartilage does not occur from sea water.

3. The ionic strength of the solution appears to be an important factor governing calcification.

4. Sodium ion inhibits calcification slightly more than potassium ion.

5. Magnesium ion appears to exercise a marked specific inhibitory effect upon calcification.

BIBLIOGRAPHY

(1) SHIPLEY, P. G.: *Johns Hopkins Hosp. Bull.*, 1924, xxxv, 304.

(2) SHIPLEY, KRAMER AND HOWLAND: (a) *Trans. Am. Ped. Soc.*, 1925, xxxvii, 36. (b) *Am. J. Dis. Ch.*, 1925, xxx, 37. (c) *Biochem. J.*, 1926, xx, 379. (d) SHIPLEY AND HOLT: *Johns Hopkins Hosp. Bull.*, 1927, xl, 1. (e) KRAMER, SHELLING AND ORENT: *Proc. Soc. Exp. Biol. and Med.*, 1926, xxiv, 240.

(3) HOLT, LAMER AND CHOWN: *J. Biol. Chem.*, 1925, lxiv, 509.

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